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Substrate Incorporation into Lipids and Proteins in Human Liver Slices

An experimental study with special reference
to dyslipoproteinemic conditions

By Håkan Stakeberg

Acta Medica Scandinavica

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By

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This thesis is based on the following papers

- I Stakeberg H. Gustafson A. & Scherstén T : Incorporation rate of leucine into proteins in human liver slices. Submitted for publication in Europ J Clin Invest
- II Stakeberg H. Lundborg H & Scherstén T : Incorporation rate in vitro of precursors into hepatic lipids and proteins in patients with extrahepatic cholestasis. Submitted for publication in Europ J Clin Invest
- III Stakeberg H. & Scherstén T : Substrate incorporation into hepatic lipids and proteins in vitro in patients with prebeta hyperlipoproteinemia. Submitted for publication in Scand. J Clin Lab Invest
- IV Cahlin E. Jönsson Jane Persson B. Stakeberg H. Björntorp P. Gustafson A. & Scherstén T : Sucrose feeding in man. Effects on substrate incorporation into hepatic triglycerides and phosphoglycerides in vitro and on removal of intravenous fat in patients with hyperlipoproteinemia. Scand J Clin Lab Invest 32: 21, 1973

These papers will be referred to by their Roman numbers

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In 1950 J W Gofman (35) suggested that coronary heart disease (CHD) was associated with high plasma levels of certain lipoprotein density classes. Since then retrospective (17 18 22 27 40) as well as prospective studies (12) have disclosed that several types of hyperlipoproteinemia are risk factors in the development of CHD (for reviews cf 93 13).

A great deal of work has been done to classify these hyperlipoproteinemic conditions and to elucidate their etiological mechanisms (for reviews cf 32 56).

Generally speaking an increased concentration of circulating lipoproteins may be the result of an increased production rate, a decreased removal rate, or a combination of both. The liver is the primary site of the synthesis and secretion of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) (46). The removal of VLDL, on the other hand, is at least partly dependent on the activity of adipose tissue lipoprotein lipase (75). It is now accepted that in patients with endogenous hyperlipoproteinemia the VLDL concentration (often referred to as prebeta lipoproteins) in the plasma may be increased and maintained after sucrose feeding (for reviews cf IV). Most information on the effect of dietary carbohydrates on the lipid metabolism, however, is derived from studies in animals. Furthermore, the kinetic behavior of plasma and liver lipids has in many instances indicated marked species differences (2 28 39 57) and many results obtained from animal studies cannot be extrapolated directly to man.

Most investigations in human beings on the mechanisms of dyslipoproteinemic conditions have applied a kinetic approach. Thus either isotope-labeled glycerol or fatty acids have been administered intravenously as precursors for the endogenous hepatic lipoprotein synthesis and secretion into the blood, after which the decay of lipoprotein radioactivity has been followed. The results of these studies have been conflicting, however, partly because of methodological problems. This has recently been critically reviewed by Nikkilä *et al* (55 56).

So far, a great deal of knowledge has been gained about the production and removal of plasma lipids. On the other hand, only sparse information with respect to the metabolism of the protein part of the lipoprotein is available. It has been suggested that the synthesis rate of carrier proteins (apoproteins) may be of importance for at least the VLDL secretion rate from the liver (14 32 46 62).

This study was designed to obtain more direct information regarding the contributive role of the liver in the genesis of different dyslipoprotein-

mic conditions in man

Extrahepatic cholestasis in man is associated with well-defined changes in the plasma lipoprotein pattern (33 69 75 76 77 83 90) These changes are suggested to be at least partly secondary to a changed hepatic lipid- and protein metabolism as indicated by studies in animals (47 73 92) and in man (86) Therefore patients with this disorder were studied and served as models for a dyslipoproteinaemic condition with a liver metabolic involvement The main purpose of this investigation however was to characterize and evaluate the role of the hepatic protein and lipid synthesis rate in the production of increased plasma concentrations of VLDL in patients with prebeta hyperlipoproteinaemia

An in vitro technique with human liver slices to determine the tri glyceride and phosphoglyceride synthesis capacity has previously been described by Nilsson & Scherstén (58) from this laboratory Another aim of this study therefore was to work out and characterize a method to measure the capacity of human liver slices to incorporate amino acids into proteins as an expression of the protein synthesis rate

Anesthesia and liver biopsy

The patients were premedicated one hour before the introduction of general anesthesia by means of diazepam and atropine. Anesthesia was given as hexobarbital (Evipen^R), nitrous oxide, oxygen and succinyl choline. Liver tissue for *in vitro* studies was obtained by a biopsy technique according to Björntorp et al. (9). The technique was described in detail by Gottfries (36). The biopsies were performed immediately after the abdominal cavity was opened and the excised liver tissue was placed in ice-cold buffer solution and rapidly brought to the laboratory (< 5 min.)

Determination of the incorporation rate of leucine into proteins in human liver slices (I, II, III)

Different preparations to determine the amino acid incorporation rate *in vitro* into hepatic proteins in experimental animals, e.g. subcellular systems (44, 61), suspensions of free cells (82), liver slices (20, 21, 68) and perfused liver (45, 53) have been described. As a very complicated sequence of reactions, such as the protein synthesis is studied, it is desirable to preserve the integrity of the liver tissue as much as possible. For obvious reasons, the organ preparation representing the highest degree of integrity, the perfused liver, could not be chosen for studies in man. Moreover, the tissue slice technique gives, if not quantitatively, so at least semiquantitatively, the same information provided that the basic conditions with respect to the influence of the substrate composition and concentration are well characterized (68).

Using the technique described in detail in Paper I, it was possible to determine with sufficient accuracy the incorporation rate of leucine into soluble proteins in human liver tissue slices. It was demonstrated that zero order kinetics were obtained for at least four hours when 500 mg liver slices were incubated in a "complete amino acid" medium. The incorporation rate of leucine into proteins revealed saturation kinetics at a concentration four times that of the serum of a complete amino acid mixture in the incubation medium. In this system, we found an intracellular leucine concentration of the same magnitude as in the medium (based on calculations of determined inulin space and diffusible water). According to Peters et al. (68) and Mortimore et al. (53), as reviewed in Paper I, the specific activity of the precursor amino acid in the medium may be valid for the calculation of the protein synthesis rate under these conditions.

Addition of insulin to the medium (100 mU/ml) increased the incorporation rate of leucine by 32 % in three consecutive experiments, whereas

supplementation with glucose (5.5 mmol/l) or fructose (5.5 mmol/l) caused no change or an inhibition of the incorporation rate of leucine respectively. These findings are in good agreement with previous studies in experimental animals (11, 51, 54) and indicate that the technique described is adequate to reveal changes in the incorporation rate of leucine into liver proteins in man at least as accurately as previously described for experimental animal liver tissue preparations.

Leucine is metabolized in the liver to a low extent. Thus, label was found in CO_2 , aceto-acetate and in lipids. This incorporation into various metabolites represented a small fraction, however, which agrees with other reports (26, 68). This finding probably reflects the relative inability of the liver to degrade branched-chained amino acids, which in turn makes them suitable as labeled precursors in *in vitro* studies of the present kind.

The standard error of the method for the incorporation rate of leucine into proteins, calculated on the basis of 38 duplicates, was ± 9.5 per cent, and for the isolation of proteins from liver tissue ± 4 per cent.

Determination of the incorporation rates of glycerol and fructose into glycerides and into glycogen in human liver slices (II, III, IV).

The technique used for this purpose was worked out in this laboratory several years ago (58). For details concerning the incubation procedure see Paper IV.

The basic incubation medium consisted of 3 per cent albumin in Krebs Ringer phosphate buffer, pH 7.4. Palmitic acid, glycerol and fructose were added to this medium to obtain the appropriate concentration of these substances in each individual experiment. As radioactive precursors for the lipid synthesis, glycerol $1\text{-}^{14}\text{C}$ or fructose- $6\text{-}^{14}\text{C}$ were used. The incubation time was four hours. The incorporation time curve for glycerol and fructose into phospholipids and triglycerides was linear for at least five hours (58). After incubation, the liver slices were submerged to 10 ml chloroform-methanol (2/1 v/v). After 22 hours, the extract was washed with 10 ml of 0.05 M KCl by a modification of the method of Folch et al. (30).

The standard error for the incorporation rate of glycerol into lipids was determined on the basis of a series of duplicates and was found to be ± 9 per cent.

The lipid synthesis was calculated from the specific activity of the precursor in the medium and recovered radioactivity in the glycerides and expressed as nmol incorporated glycerol or fructose per gram liver per hour. Analytical procedures.

Separation of different lipids in the chloroform-methanol extracts from plasma and liver tissue was performed by thin-layer chromatography (TLC).

according to Gloster & Fletcher (34) The determination of lipid phosphorus was performed according to the Bartlett method (5) as modified by Svennerholm & Vanier (89) (standard error of the method 5.0 per cent) Glyceride-glycerol was determined as described by Carlson (19) (error 3.3 per cent) cholesterol according to Sperry & Webb (85) (error 10.4 per cent) glucose according to Levin et al (50) and glycogen according to van der Vies (94) (error 4.2 per cent) IRI insulin was determined radio-immunochemically according to Hales & Randle (41) using assay kits from Amersham (insulin immuno assay kit the Radiochemical Center Amersham, Buckingham England) (error 7.1 per cent) Protein was quantitated according to Lowry et al (49) (error per cent)

The radioactivity was determined in a Packard Tri-Carb (3320) liquid scintillation spectrometer Correction for quenching was performed by the external standard method.

Experimental procedures in patients on a carbohydrate-enriched diet (III-IV)

All patients on a carbohydrate-enriched diet were hospitalized for 20 days and the patient group designated as non-sucrose-fed controls for two days prior to the gallbladder operation In the morning on the day after admission after an overnight fast capillary blood and heparinized venous blood samples were drawn for subsequent determination of blood glucose (50) basal plasma insulin (41) plasma cholesterol (85) and triglycerides (19) and for lipoprotein electrophoresis (71) A glucose tolerance test was performed the same day as described in detail in Paper IV

In the morning of the second day the intravenous fat (Intralipid[®]) tolerance test (10) was performed with the patient in the fasting state Just before the tolerance test a percutaneous biopsy of adipose tissue was taken from the gluteal region (66) The samples obtained were used to determine the lipoprotein lipase activity (6)

The patients were given 200 g sucrose per day from the third day through the 17 th day The ordinary hospital diet contained an average of 2500 Kcal distributed as 400 Kcal of protein 900 Kcal of fat and 1100 Kcal of carbohydrates mainly as starch With the aid of a dietician approximately 800 Kcal (160 Kcal protein 290 Kcal fat and 350 Kcal carbohydrates) of the daily food intake was replaced by 200 g of sucrose The sucrose solution kindly supplied by the Bechtem Company London England was flavored and dispensed in 100 g bottles

Throughout the sucrose feeding period blood samples were taken every second day to determine plasma cholesterol and triglycerides After the sucrose feeding period but before the gallbladder operation a second glucose tolerance test was performed on the 17 th day The fat tolerance test with a concomitant determination of the adipose tissue lipoprotein lipase activity

were repeated on the 18 th day. These determinations were performed under the same conditions as before the sucrose feeding period.

Statistical methods (I II III IV)

In the comparison between different groups Student's t test was used. For pair differences Wilcoxon's test as described by Siegel (84) was used. Linear regressions were calculated according to the method of least squares. Standard procedures were also used to calculate correlation coefficients. T tests were applied to check the significance of regression coefficients. Standard error of the methods was calculated from duplicates using the following formulae:

$$SE = \sqrt{\frac{\sum d_i^2}{n}}$$

where d_i is the difference between duplicate measurements. The error was then expressed as a percentage of the mean of all determinations.

CLINICAL MATERIAL

The clinical material consisted of 105 subjects 38 men and 67 women aged 20-86 years. All patients except those with extrahepatic cholestasis (II) had a normal liver function according to the following criteria: serum bilirubin < 1.0 mg/100 ml; thymol turbidity < 0.10 ; alkaline phosphatase (EC 3.1.3.1) < 10 units (Buch); L-aspartate 2-oxoglutarate (EC 2.6.1.1) < 20 units (NAD/NADH₂).

None of the patients had any known disease apart from the gallbladder disease and in pertinent cases: prebeta hyperlipoproteinemia (except Patient No. 1, Paper III, who had slight maturity onset diabetes mellitus treated with sulfonylurea). At the time of the study or shortly before, none was taking any drug known to affect the liver function. Patients with known or suspected alcoholic problems and women taking oral contraceptive steroids were excluded from the study.

The patients were divided into different groups on the basis of their initial plasma lipoprotein pattern according to Fredrickson & Lees (6, 31). The plasma lipoprotein type was determined from repeated plasma lipid analyses, lipoprotein electrophoresis on agarose gel (71) and alphalipoprotein cholesterol determinations (6). Based on these analyses, determinations of prebeta lipoprotein and beta lipoprotein cholesterol were performed according to Gustafson et al. (40). The lipoprotein values were used for the subsequent lipoprotein typing. The presence at repeated analyses of prebeta lipoproteins at agarose gel electrophoresis concomitant with > 35 mg/100 ml of prebeta lipoprotein cholesterol were used as definition of prebeta hyperlipoproteinemia. The corresponding upper limit for beta lipoprotein cholesterol was set at 200-220 mg/100 ml, dependent on the patient's age and sex.

The group with prebeta hyperlipoproteinemia (n = 21) comprised 6 patients with hyperlipoproteinemia Type II B and 15 patients with Type IV. All controls were admitted to the hospital for an operation for uncomplicated gallbladder disease. The plasma triglyceride and cholesterol concentrations in the control group were not different from those of middle-aged men and women randomly selected from the same city (8).

Fifteen of the patients studied had extrahepatic cholestasis (II). Clinical data on the time of the liver biopsy and the blood sampling are reported in detail in Paper II. In all patients the diagnosis was confirmed at laparotomy and in the five tumor patients by means of microscopy. No detectable metastasis in lymph nodes or liver was found in any of these patients. Patients Nos. 1, 3 and 11 had localized carcinoma of the head of the pancreas strangulating the common bile duct, while patients Nos. 2 and 14 had small

isolated bile duct carcinomas

Consent was obtained from all patients on the carbohydrate-enriched dietary regimen. No complications in connection with the liver biopsies or the other procedures were encountered.

Incorporation rate of leucine into hepatic proteins *in vitro* in normolipoproteinemic controls (I)

The incorporation rate of leucine into proteins in liver slices from 42 normolipidemic gallstone patients was studied with the technique described in detail in Paper I. No sex differences were found. In subjects older than 60 years we found a higher ($P < 0.025$) incorporation rate of leucine into proteins than in subjects below the age of 60. Since the protein content in the liver tissue from these subjects was approximately the same as in the younger ones, this finding may be interpreted as a more rapid hepatic protein turnover rate in older subjects. This would be in line with the high lysosomal enzyme activity in aging human liver tissue (7) and is in agreement with the finding of an increased synthesis rate of albumin *in vivo* in the old rat (64).

Provided the incorporation rate of leucine into liver proteins can be taken as an expression of protein synthesis, it is possible to calculate the synthesis rate by dividing the amount of leucine incorporated into hepatic proteins by the mass fraction of leucine in liver proteins 0.08 (w/w) (52). This will give a synthesis rate of 0.100 mg protein per hour per gram liver tissue, which is roughly 1/10 of the corresponding synthesis rate for rat liver *in vitro* (68). Our results might indicate a protein turnover corresponding to the half life of soluble liver proteins of 3.4 days *in man*. It has to be emphasized, however, that the protein synthesis rate determined *in vitro* in rat liver slices has been found to be 1/5 - 1/10 of the rate determined *in vivo* (68).

It might be questioned whether the liver tissue used in the protein synthesis experiments can be considered normal. Sumzel et al. (87) reported a higher triglyceride content (but no differences of phospholipids and cholesterol) in liver tissue from patients with gallstone disease than in patients with peptic ulcer. Nilsson & Scherstén (58) however found no differences in the lipid synthesis pattern in liver slices from patients with diseases as different as gallstone disease, gastric disease, and renal calculus. So far this seems to hold true also for the protein synthesis. This supports the assumption that the gallstone disease of the patients in the present study did not influence the results, especially not the comparison between the different patient groups.

Substrate incorporation into hepatic lipids and proteins *in vitro* in extrahepatic cholestasis (II)

In liver tissue from fifteen patients with extrahepatic cholestasis we found an increased concentration ($P < 0.025$) of phosphoglycerides and pro-

teins compared with normolipidemic gallstone patients. The triglyceride concentration was equal while the glycogen concentration was decreased ($P < 0.05$) in these patients. The changed lipid and protein concentrations could not be ascribed to a change in the hepatic water content which was equal in the two groups.

The incorporation rate of glycerol into total phosphoglycerides ($P < 0.01$) as well as into the choline phosphoglycerides ($P < 0.05$) and ethanolamine phosphoglycerides ($P < 0.01$) was increased in cholestatic liver tissue compared with controls. On the other hand, liver tissue from patients with cholestasis incorporated glycerol into triglycerides at the same rate as controls.

The incorporation rate of leucine into liver proteins was increased ($P < 0.015$) in the cholestatic liver tissue which also had a high protein content ($P < 0.025$) compared with the controls. These findings may be interpreted as indicating an enhanced synthesis of phosphoglycerides and proteins in cholestatic liver tissue.

This interpretation was supported by the higher concentrations of phosphoglycerides and proteins in the cholestatic liver slices. The final interpretation of these data should be made with caution, however, since the possibility of a changed specific activity in the immediate precursor pool can not be ruled out completely. The fact that the incorporation rate of glycerol into triglycerides was unchanged concomitant with a significant increase in the incorporation rate into phosphoglycerides does not support the hypothesis of a changed specific activity in the α -glycerophosphate pool. Moreover, the free leucine concentration was found to be almost equal (600-700 $\mu\text{mol/l}$) in cholestatic and normal liver tissue.

An enhanced synthesis of phosphoglycerides in liver tissue under cholestatic conditions may be explained by an increased bile acid concentration in the liver. Grein et al. (38) have shown that biliary obstruction causes an increased cholic acid concentration comparatively early, whereas chenodeoxycholic acid increased only when the cholestasis was protracted. A stimulating effect of bile acids on the lecithin synthesis seems to be well established in different species (3, 42, 59, 79, 81, 88). The mechanism of the effect of bile acids on the phospholipid synthesis is not known, however, we have previously shown (15) that in vitro addition of bile acids to incubations with liver slices caused a shunt in glycerol into lecithin synthesis pathways. This finding provided support for the hypothesis that bile acids in some way directly affect the hepatic synthesis of lecithin. This hypothesis is also supported by the finding of Kennedy & Heiss (48) that the lecithin synthesis in rat liver preparations was stimulated in the presence of detergents such as bile acids.

The mechanism behind the increased protein synthesis in cholestatic liver tissue is not easy to explain. An increased incorporation rate of amino acids into hepatic proteins in experimentally induced cholestasis in rats has been found (73). Morphometric analyses of electron micrographs of liver tissue from patients with cholestasis have shown an increased surface area of both the rough and smooth endoplasmic reticulum (37). This probably indicates a 'hypertrophy' in that part of the cell which is mainly responsible for the protein synthesis.

Our finding of a significant correlation between the incorporation rate of glycerol into phosphoglycerides and the incorporation rate of leucine into proteins in cholestatic liver may suggest a common mechanism for the enhancement of both these processes. In one series of experiments with control liver slices, however, glycocholic acid or glycochenodeoxycholic acid was added to the incubation medium (final concentration 0.05 mmol/l). Neither one of these primary bile acids influenced the incorporation rate of leucine into proteins in contrast to the effect of the bile acids on the incorporation rate of glycerol into phosphoglycerides (79).

Another contributive mechanism to the enhanced incorporation of glycerol into fatty acids and leucine into proteins in cholestatic liver tissue has to be considered. Thus, the reduced form of nicotinamide-adenine nucleotide monophosphate has been found to enhance the synthesis of amino acids and proteins (95). In a previous work, Scherstén et al. (78, 80) found that the ratio between reduced and oxidized nucleotides was considerably increased in cholestatic liver tissue. Thus, their findings are in line with those of the present study.

As pointed out in the introduction, extrahepatic cholestasis can be looked upon as a model system for dyslipoproteinemic conditions. Whether the changed plasma lipoprotein pattern in cholestasis is related to the changed liver tissue metabolism can not be unequivocally determined on the basis of the present results. It appears tempting, however, to assume that the increased plasma lecithin concentration in this condition may be caused at least partly by the enhanced hepatic synthesis. An additional cause may be a diminished lecithin secretion into bile. The appearance of the abnormal lipoprotein-X may be associated with such changes in the protein and phospholipid synthesis in the liver.

Effects of sucrose feeding in patients with prebeta hyperlipoproteinemia (III, IV)

Four patient groups were studied as described in detail in Papers III and IV. They are referred to as non-sucrose-fed controls (n = 36), non-sucrose-fed patients with prebeta hyperlipoproteinemia (n = 8), sucrose-fed controls.

(n 15) and sucrose-fed patients with prebeta hyperlipoproteinemia (n 13)

In the normolipoproteinemic patients sucrose feeding produced an increase in the plasma triglycerides ($P < 0.001$) a transient decrease in the plasma cholesterol ($P < 0.005$) an increase in basal insulin ($P < 0.01$) concomitant with an increased lipoprotein lipase activity ($P < 0.01$) Only basal insulin increased significantly in the sucrose-fed patients with pre-beta hyperlipoproteinemia

Compared with non-sucrose-fed controls the liver triglyceride content was increased in sucrose-fed controls ($P < 0.05$) as well as in non-sucrose-fed ($P < 0.05$) and sucrose-fed patients with prebeta hyperlipoproteinemia ($P < 0.05$) An increased incorporation rate of glycerol into hepatic triglycerides was found in the above-mentioned groups except for the sucrose-fed patients with prebeta hyperlipoproteinemia The higher incorporation rate of precursors into triglycerides was taken as an indication of an increased hepatic triglyceride synthesis in the sucrose-fed controls and the non-sucrose-fed patients with prebeta hyperlipoproteinemia The increased liver triglyceride content supports the validity of this finding An additional fact which supported the finding was the significant correlation between the incorporation rate of precursors into hepatic triglycerides and the increment of plasma triglycerides on the seventh day of sucrose feeding in the sucrose-fed controls as well as the significant correlation between the hepatic triglyceride synthesis and the plasma triglyceride concentration in the non-sucrose-fed patients with prebeta hyperlipoproteinemia

In the sucrose-fed patients with prebeta hyperlipoproteinemia the mean plasma triglycerides as well as the mean incorporation rate of precursors into hepatic triglycerides showed a wide range of values This may be an expression of heterogeneity within this series of biochemically typed patients with prebeta hyperlipoproteinemia It should be kept in mind that this patient group in addition to comprising patients with Type IV also included 6 patients with Type II B Furthermore it remains to be shown that patients with Type IV hyperlipoproteinemia constitute a metabolically homogenous population (70)

In the sucrose-fed patients with prebeta hyperlipoproteinemia the mean of the incorporation rate of radioactive precursors into triglycerides was moderately increased but less than in the sucrose-fed controls Caution should be exercised in the final interpretation of these data however since the possibility of a dilution of the label by an expanded precursor pool of α -glycerophosphate can not be completely ruled out The facts that the incorporation rates of glycerol and fructose into triglycerides showed an increase together with a highly significant decrease in the incorporation rate into phosphoglycerides however do not support the hypothesis of a dilution of the

immediate precursor pool. Furthermore, these findings are in agreement with conclusions drawn from other investigations in man (24-29, 70).

The finding of a lower incorporation rate of glycerol as well as of fructose into hepatic choline phosphoglycerides in the sucrose-fed patients with pre-beta hyperlipoproteinemia seems to be further supported by the lower liver content of phosphoglycerides in these patients. These data are also in agreement with our observation of a low choline phosphoglyceride concentration in the bile from the same patient group (16). The data also concur with the recent finding of Park et al. (65) that in high carbohydrate-fed rats the choline phosphoglyceride level in the rough microsomal fraction of liver homogenates was significantly decreased and that the linoleic and arachidonic acids were virtually absent in the phospholipids of this fraction. Recently Alling et al. (1) showed that sucrose feeding rapidly caused a significant decrease in the proportion of linoleic acid in liver plasma and bile choline phosphoglycerides, indicating that sucrose feeding inhibits the cytidindiphosphate choline pathway (Kennedy's pathway) of the choline phosphoglyceride synthesis.

Thus, this decreased choline phosphoglyceride synthesis seems to be provoked by sucrose feeding, since non-sucrose-fed patients with pre-beta hyperlipoproteinemia had a normal incorporation rate of precursors into hepatic glycerides as well as a normal hepatic phosphoglyceride content. Pre-beta hyperlipoproteinemia is not characterized by low plasma phosphoglycerides or plasma choline phosphoglycerides (60), but the VLDL in Type IV have a lower specific density and a higher Sf value (4) and would therefore have a higher triglyceride/phosphoglyceride ratio than that of normolipoproteinemic subjects.

The low incorporation rates of glycerol and fructose into phosphoglycerides are most likely not an effect of the conditions chosen for the in vitro incubation. Neither an increase in the fatty acid amount in the system (58-63) nor the use of another fatty acid substrate like oleic acid (43) or linoleic acid (15) would be expected to increase the incorporation into phosphoglycerides. The addition of insulin to the system would be expected to influence the incorporation into phosphoglycerides only to a moderate extent (91).

The incorporation rate of leucine into hepatic proteins was equal in non-sucrose-fed controls (n = 20) and non-sucrose-fed patients with pre-beta hyperlipoproteinemia (n = 9). Three of the sucrose-fed controls (n = 4) incorporated leucine into proteins at a rate above the 95 per cent confidence level of that for non-sucrose-fed controls. This may indicate an insulin effect on the hepatic protein synthesis capacity (1-51) which has been found also after long-term glucose feeding in rats (23). No change in the incorporation rate of leucine into hepatic proteins in sucrose-fed patients with pre-beta

(n = 15) and sucrose-fed patients with prebeta hyperlipoproteinemia (n = 13)

In the nonlipoproteinemic patients sucrose feeding produced an increase in the plasma triglycerides ($P < 0.001$) a transient decrease in the plasma cholesterol ($P < 0.005$) an increase in basal insulin ($P < 0.01$) concomitant with an increased lipoprotein lipase activity ($P < 0.01$) Only basal insulin increased significantly in the sucrose-fed patients with pre-beta hyperlipoproteinemia.

Compared with non-sucrose-fed controls the liver triglyceride content was increased in sucrose-fed controls ($P < 0.05$) as well as in non-sucrose-fed ($P < 0.05$) and sucrose-fed patients with prebeta hyperlipoproteinemia ($P < 0.05$) An increased incorporation rate of glycerol into hepatic triglycerides was found in the above-mentioned groups except for the sucrose-fed patients with prebeta hyperlipoproteinemia The higher incorporation rate of precursors into triglycerides was taken as an indication of an increased hepatic triglyceride synthesis in the sucrose-fed controls and the non-sucrose-fed patients with prebeta hyperlipoproteinemia The increased liver triglyceride content supports the validity of this finding An additional fact which supported the finding was the significant correlation between the incorporation rate of precursors into hepatic triglycerides and the increment of plasma triglycerides on the seventh day of sucrose feeding in the sucrose-fed controls as well as the significant correlation between the hepatic triglyceride synthesis and the plasma triglyceride concentration in the non-sucrose-fed patients with prebeta hyperlipoproteinemia.

In the sucrose-fed patients with prebeta hyperlipoproteinemia the mean plasma triglycerides as well as the mean incorporation rate of precursors into hepatic triglycerides showed a wide range of values This may be an expression of heterogeneity within this series of biochemically typed patients with prebeta hyperlipoproteinemia It should be kept in mind that this patient group in addition to comprising patients with Type IV also included 6 patients with Type II B Furthermore it remains to be shown that patients with Type II hyperlipoproteinemia constitute a metabolically homogenous population (70)

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SUMMARY

- 1 Liver tissue from normolipidemic gallstone patients older than 60 years incorporated leucine into hepatic proteins at a higher rate than liver tissue from younger individuals indicating a higher protein turnover rate in the older patients
- 2 Cholestatic liver tissue contained more proteins and phosphoglycerides and incorporated precursors into proteins and phosphoglycerides at a higher rate than presumably normal liver tissue The incorporation rate of glycerol into hepatic phosphoglycerides correlated significantly with the plasma phosphoglyceride concentration
- 3 Liver tissue from patients with prebeta hyperlipoproteinemia on an ordinary diet contained more triglycerides and incorporated glycerol into triglycerides at a higher rate than liver tissue from normolipidemic gallstone patients The incorporation rate of precursors into hepatic triglycerides correlated significantly with the plasma triglyceride concentration indicating an increased hepatic triglyceride synthesis rate
- 4 After sucrose feeding for 14 days liver tissue from patients with prebeta hyperlipoproteinemia had a lower content of phosphoglycerides and incorporated precursors into phosphoglycerides at a lower rate compared with non-sucrose-fed patients with prebeta hyperlipoproteinemia indicating a phosphoglyceride synthesis defect provoked by the sucrose feeding
- 5 Sucrose-fed controls showed an increased incorporation rate of glycerol and fructose into hepatic triglycerides in vitro which correlated with the increase in the plasma triglyceride concentration indicating an increased triglyceride synthesis rate
- 6 The fractional elimination rate of exogenous fat decreased after sucrose feeding in controls as well as in patients with prebeta hyperlipoproteinemia indicating a removal defect for at least exogenous fat
- 7 The adipose tissue lipoprotein lipase activity was increased after sucrose feeding in controls and was inversely correlated to the plasma triglyceride concentration in controls as well as in patients with prebeta hyperlipoproteinemia.

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REFERENCES

- 1 Ailing C. Cahlin E. Scherstén T. Relationships between fatty acid patterns of serum, hepatic and biliary lecithins in man. Effect of sucrose feeding. *Biochim. Biophys. Acta* 296: 518, 1973.
- 2 Baker N. Schotz M.C. Quantitative aspects of free fatty acid metabolism in fasted rat. *J. Lipid Res.* 8: 646, 1967.
- 3 Balint J.A. Beeler D.A. Kyriakides E.C. Treble D.H. The effect of bile salts upon lecithin synthesis. *J. Lab. Clin. Med.* 77: 12, 1971.
- 4 Barter P.J. Nestel P.J. The distribution of triglyceride in subclasses of very low density plasma lipoproteins. *J. Lab. Clin. Med.* 76: 925, 1970.
- 5 Bartlett G.R. Phosphorus assay in column chromatography. *J. Biol. Chem.* 234: 466, 1959.
- 6 Beaumont J.L. Carlson L.A. Cooper G.R. Fejfar Z. Fredrickson D.S. Strasser T. Classification of hyperlipidaemias and hyperlipoproteinaemias. *Bull. Wild Hlth Org.* 43: 891, 1970.
- 7 Björkerud S. Björntorp P. Scherstén T. Lysosomal enzyme activity in human liver in relation to age of the patient and in cases with obstructive jaundice. *Scand. J. Clin. Lab. Invest.* 20: 224, 1967.
- 8 Björntorp P. Bengtsson C. Blohm G. Johnsson A. Sjöström L. Tibblin B. Tibblin G. Wilhelmsen L. Adipose tissue fat cell size and number in relation to metabolism in randomly selected middle-aged men and women. *Metabolism* 20: 927, 1971.
- 9 Björntorp P. Björkerud S. Scherstén T. Subcellular fractionation of human liver. *Biochim. Biophys. Acta* 111: 375, 1965.
- 10 Boberg J. Carlson L.A. Hallberg D. Application of a new intravenous fat tolerance test in the study of hypertriglyceridemia in man. *J. Atheroscler. Res.* 9: 159, 1969.
- 11 Bode J.C. Zelder D. Rumpelt H.J. Wittkamp W. Depletion of liver adenosine phosphates and metabolic effects of intravenous infusion of fructose or sorbitol in man and in the rat. *Europ. J. Clin. Invest.* 3: 436, 1973.

- 12 Brown D F Kinch S H Doyle J T Serum triglycerides in health and in ischemic heart disease *New Eng J Med* 273 947 1965
- 13 Brown D F Blood lipids and lipoproteins in atherogenesis *Amer J Med* 46 691 1969
- 14 Buckley I T Delahanty E J Rubinstein D The relationship of protein synthesis to the secretion of the lipid moiety of low density lipoprotein by the liver *Canad J Biochem* 46 341 1968
- 15 Cahlin E Jönsson Jane Nilsson S Scherstén T Synthesis of phospholipids and triglycerides in human liver slices III Influence of bile acids choline and linoleic acid *Scand J Clin Lab Invest* 29 109 1972
- 16 Cahlin E Jönsson Jane Nilsson S Scherstén T Biliary lipid composition in normolipidemic and prebeta hyperlipoproteinemic gallstone patients Influence of sucrose feeding of the patient on the biliary lipid composition *Scand J Gastroent* 8 449 1973
- 17 Carlson L A Serum lipids in men with myocardial infarction *Acta Med Scand* 167 399 1960
- 18 Carlson L A Wahlberg F Serum lipids intravenous glucose tolerance and their interrelation studied in ischemic cardiovascular disease *Acta Med Scand* 180 307 1966
- 19 Carlson L A Determination of serum glycerides *Acta Soc Med Upsalien* 64 208 1959
- 20 Clemens M J Korner A Amino acid requirement for the growth hormone stimulation of incorporation of precursors into protein and nucleic acids of liver slices *Biochem J* 119 629 1970
- 21 Crawhall J C Purkiss P Transport of methionine and proline by rat liver slices and the effect of certain hormones *Biochem J* 136 15 1973
- 22 Dyerberg J Bang H O Hansen I A Plasma lipids and lipoproteins in patients 1 years after an acute myocardial infarction *Scand J Clin Lab Invest Suppl* 110 54 1969

- 23 Eaton R.P Kipnis D.M Effect of glucose feeding on lipoprotein synthesis in the rat *Amer J Physiol* 217 1153 1969
- 24 Eaton R.P Synthesis of plasma triglycerides in endogenous hypertriglyceridemia *J Lipid Res* 12 491 1971
- 25 Eder H.A. Russ H.M. Pritchett R.A.R. Wilber M.M. Barr D.P Protein-lipid relationships in human plasma In biliary cirrhosis obstructive jaundice and acute hepatitis *J Clin Invest* 34 1147 1955
- 26 Elwyn D.H. Parikh H.C. Schoemaker W.C. Amino acid movements between gut liver and periphery in unanesthetized dogs *Amer J Physiol* 215 1260 1968
- 27 Enger S.C. Ritland E. Serum lipoprotein (LP) pattern in myocardial infarction *Scand J Clin. Lab Invest Suppl* 110 56 1969
- 28 Farquhar J.W. Gross R.C. Wagner R.M. Reaven G.M. Validation of an incompletely coupled two-compartment non-recycling catenary model for turnover of liver and plasma triglyceride in man *J Lipid Res* 6 119 1965
- 29 Fine M. Michaels G. Shah J. Chai B. Pukys G. Kinsell L. The incorporation of ^{14}C from uniformly labeled glucose into plasma triglycerides in normals and hyperglyceridemics *Metabolism* 11 893 1962
- 30 Felch J. Lees M. Sloane-Stanley G.H. A simple method for the isolation and purification of total lipids from animal tissues *J Biol Chem.* 226 497 1957
- 31 Fredrickson D.S. Lees R.S. A system for phenotyping hyperlipoproteinemia *Circulation* 31 321 196
- 32 Fredrickson D.S. Levy R.I. Lees R.S. Fat transport in lipoproteins An integrated approach to mechanisms and disorders *New Eng J Med* 276 34 1967
- 33 Gjone B. Orning O.M. Plasma phospholipids in patients with liver disease A quantitative thin-layer chromatographic study *Scand J Clin Lab Invest* 18 209 1966
- 34 Gloster J. Fletcher R.F. Quantitative analysis of serum lipids with thin-layer chromatography *Clin Chim. Acta* 13 235 1966

- 35 Gofman J W Role of lipids and lipoproteins in atherosclerosis Science 111 166 1950
- 36 Gottfries A. Studies on acute cholecystitis A clinical and experimental study in humans and in animals with special reference to liver function and pathogenesis Acta Chir Scand Suppl 393 1968
- 37 Grainger J M. Scheuer P J : Quantitative changes in liver cell organelles in cholestasis J Path 106 pv 1972
- 38 Greim, H. Trölzsch D Czygan P Rudick J Hutterer F Schaffner F Popper H Mechanism of cholestasis II Bile acids in human livers with or without biliary obstruction Gastroenterology 63 846 1972
- 39 Gross R.C Eigenbrodt B H Farquhar J W Endogenous triglyceride turnover in liver and plasma of the dog J Lipid Res II 114 1967
- 40 Gustafson A. Elmfeldt D Wilhelmson L Tibblin G Serum lipids and lipoproteins in man after myocardial infarction compared with representative population sample Circulation 46 709 1972
- 41 Hales C N Randle P J Immunoassay of insulin with insulin antibody precipitate Lancet I 200 1963
- 42 Heath T Caple I W Redding P M. Effect of the enterohepatic circulation of bile salts on the flow of bile and its content of bile salts and lipids in sheep Quart J Exp Physiol 55 93 1970
- 43 Heimberg M Wilcox H G The effect of palmitic and oleic acids on the properties and composition of the very low density lipoprotein secreted by the liver J Biol Chem. 247 875 1972
- 44 Hochberg A. Stratsman P W Zahlten, R.N Lardy H A. Artefacts in protein synthesis by mitochondria in vitro Febs Letters 25 1 1972
- 45 Jefferson L S Korner A. Influence of amino acid supply on ribosomes and protein synthesis of perfused rat liver Biochem. J 111 703 1969
- 46 Jones A.L Ruderman N B Herrera M G Electron microscopic and biochemical study of lipoprotein synthesis in the isolated perfused rat liver J Lipid Res 8 429 1967
- 47 Kattermann R. Creutzfeldt W The effect of experimental cholestasis on the negative feedback regulation of cholesterol synthesis in rat Liver Scand J Gastroent 5 337 1970

- 48 Kennedy E P Weiss S B The function of cytidin coenzymes in the bile synthesis of phospholipids J Biol Chem 222 193 1956
- 49 Lowry O H. Rosebrough N.J. Farr L.A. Randall R J Protein measurement with the Folin phenol reagent J Biol Chem. 193 265 1951
- 50 Levin K. Linde S Determination of glucose in blood cerebrospinal fluid and urine with a new glucose oxidase reagent Svensk Läk Tidn 59 3016 1962
- 51 Manchester K L. Sites of hormonal regulation of protein metabolism In Metabolism protein metabolism. Ed E.H. Marmore Acad Press NY London Vol IV p 229
- 52 Morgan E H Peters T Jr The biosynthesis of rat serum albumin J Biol Chem. 246 3500 1971
- 53 Mortimore G B Woodside K.H. Henry J B Compartmentation of free valine and its relation to protein turnover in perfused rat liver J Biol Chem. 247 2776 1972
- 54 Mortimore G B Mordon C B Inhibition by insulin of valine turnover in liver Evidence for a general control of proteolysis J Biol Chem 245 2375 1970
- 55 Nikkila E A Control of plasma and liver triglyceride kinetics by carbohydrate metabolism and insulin Advances Lipid Res 7 63 1969
- 56 Nikkila E Kekki M. Measurement of plasma triglyceride turnover in the study of hyperglyceridemia Scand J Clin Lab Invest 27 97 1971
- 57 Nikkila E A. Pelkonen R Enhancement of alimentary hyperglyceridemia by fructose and glycerol in man Proc Soc Exp Biol Med (N.Y.) 123 91 1966
- 58 Nilsson S Scherstén T Synthesis of phospholipids and triglycerides in human liver slices Scand. J Clin Lab Invest 24 237 1969
- 59 Nilsson S Scherstén T Influence of bile acids on the synthesis of biliary phospholipids in man. Europ J Clin Invest 1 109 1970
- 60 Noel C Marcel Y L. Davignon J Plasma phospholipids in the different types of primary hyperlipoproteinemia J Lab Clin Med 79 611 1972

- 61 Nolan R.D Hogland M.B Increased protein synthesis capacity in vitro of rat liver rough endoplasmic reticulum following starvation *Biochim. Biophys Res Commun* 51 404 1973
- 62 Olson R.E Vester J.W Gursley III Davies N Longman D The effect of low protein diets upon serum cholesterol in man *Amer J Clin Nutr* 6 310 1958
- 63 Ontko J.A Metabolism of free fatty acids in isolated liver cells *J Biol Chem.* 247 1788 1972
- 64 Ove P Obermader M. Lansing A Synthesis and degradation of liver proteins in young and old rats *Biochim Biophys Acta* 277 211 1972
- 65 Park C.E Marai E Mookerjee S Phospholipid metabolism in endoplasmic reticular membranes in rats fed high-carbohydrate diet *Biochim Biophys Acta* 270 50 1972
- 66 Persson B Björntorp P Hood B Lipoprotein lipase activity in human adipose tissue I Conditions for release and relationship to triglycerides in serum. *Metabolism* 15 730 1966
- 67 Persson B Schröder G Hood III Lipoprotein lipase activity in human adipose tissue; Assay methods; Relations to the serum triglyceride level in a normolipidemic population The effect of ethyl chlorophenoxy isobutyrate *Atherosclerosis* 16 37 1972
- 68 Peters T Jr Biosynthesis of rat serum albumin VII Effects observed in liver slices *Amer J Physiol* 224 1363 1973
- 69 Phillips G.B The lipid composition of serum in patients with liver disease *J Clin Invest* 39 1639 1960
- 70 Quarfordt S.H Frank A Shames D.M. Berman M Steinberg D Very low density lipoprotein triglyceride transport in type IV hyperlipoproteinemia and the effect of carbohydrate-rich diets *J Clin Invest* 49 2181 1970
- 71 Rapp W Kahlke W Lipoprotein-Elektrophorese in Agarose gel *Clin Chim. Acta* 19 493 1968
- 72 Reaven, G.M Hill D.B Gross R.C Farquhar J.W Kinetics of triglyceride turnover of very low density lipoproteins of human plasma *J Clin Invest* 44 186 1965

- 73 Richter E, Grün M, Kühn H A. Proteinsynthese und Ikterus bei der ANIT-cholestase der Ratte. *Verh. Deutsch. Ges. Inn. Med.* 75: 367, 1969
- 74 Ritland S, Blomhoff J P, Elgjo K, Gjone H. Lipoprotein-X (LP-X) in liver disease. *Scand. J. Gastroent.* 8: 155, 1973
- 75 Robinson E S. The function of the plasma triglycerides in the fatty acid transport. In: *Lipid Metabolism*, vol. 18 of *Comprehensive Biochemistry* (eds. Florkin M, Stot. E.N.) p. 51. Elsevier, Amsterdam, 1970
- 76 Ross A, Murphy G.M., Wilkinson P A, Mills G L, Sherlock H. Occurrence of an abnormal lipoprotein in patients with liver disease. *Gut* 11: 1035, 1970
- 77 Samsioe G, Lundborg H, Ivarsson S, Gustafson A. On the occurrence of an abnormal serum lipoprotein (LP-X) in different conditions of cholestasis. *Medicinsk Riksstämman*, Stockholm, 1971
- 78 Scherstén T. Metabolic differences between hepatitis and cholestasis in human liver. In: *Progress in Liver Diseases*, vol. IV, eds. H. Popper & H. Schaffner. Grune & Stratton, Inc. New York, 1972, p. 133
- 79 Scherstén T. Bile acids as a determining factor for synthesis and secretion of human bile lecithin. *Helv. Med. Acta* 37: 161, 1973
- 80 Scherstén T, Björntorp P, Björkerud S, Sæds S, Ekholm R. Liver cell function in infectious hepatitis in man. *Acta Hepatosplen.* 17: 375, 1970
- 81 Scherstén T, Nilsson S, Cahlin E. Determining factors for the synthesis of biliary lecithin in man. *Europ. J. Clin. Invest.* 1: 174, 1970
- 82 Schreiber G, Schreiber Margot. Protein synthesis in single cell suspensions from rat liver. *J. Biol. Chem.* 247: 6340, 1972
- 83 Se del H, Alajupovic P, Furman R.H., McConathy W.J. A lipoprotein characterizing obstructive jaundice. II. Isolation and partial characterization of the protein moieties of low density lipoproteins. *J. Clin. Invest.* 49: 2396, 1970
- 84 Siegel S. *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill, New York, 1956

- 85 Sperry W.M. Webb N A revision of the Schoenheimer Sperry method for cholesterol determination J Biol Chem. 187 97 1950
- 86 Stakeberg H Gustafson A. Samsioe H Scherstén T Synthesis of proteins and glycerides in liver tissue from patients with cholestasis Digestion 6 254 1972
- 87 Sunzel H Martinsson A Hood B The lipid content of the human liver A comparison between patients with gallstone disease and peptic ulcer Metabolism 13 1469 1964
- 88 Swell L. Bell C.C. Jr Enterman C Bile acids and lipid metabolism. III Influence of bile acids on phospholipids in liver and bile of the isolated perfused dog liver Biochim. Biophys. Acta 164 278 1968
- 89 Svennerholm, L. Vanier M.T The distribution of lipids in the human nervous system. II Lipid composition of human fetal and infant brain Brain Res 47 457 1972
- 90 Switzer S Setenstain L Plasma lipoproteins in liver disease I Immunologically distinct low density lipoproteins in patients with biliary obstruction J Clin. Invest 46 1855 1967
- 91 Topping D L. Mayes P.A. The immediate effects of insulin and fructose on the metabolism of the perfused liver Biochem J 126 295 1972
- 92 Weis H.J Dietschy J.M. Presence of an intact cholesterol feedback mechanism in the liver in biliary stasis Gastroenterology 61 77 1971
- 93 Kerkö L. Can we prevent heart disease? Ann Intern Med 74 278 1971
- 94 Vlies J van der 2 methods for determination of glycogen in liver Biochem. J 57 410 1954
- 95 Wilson J D Siperstein M.D Studies on the relationship between glucose oxidation and intermediary metabolism. III The influence of pyridine nucleotides on protein synthesis J Clin Invest. 38 317 1959

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Hepatic Circulation in Hepatitis A

A Study in Young Males at Rest and during and after Supine Leg Exercise

By Per Lundbergh

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HEPATIC CIRCULATION IN HEPATITIS A

**A Study in Young Males at Rest and
during and after Supine Leg Exercise**

By
PER LUNDBERGH

STOCKHOLM 1974

To Inger
Fredrik and Elisabet

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- I Lundbergh P & Strandell T : The effect of physical exercise on the wedged and free hepatic venous pressure in normal man
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- II Lundbergh P & Strandell T Changes in hepatic circulation at rest during and after exercise in young mal with infectious hepatitis compared with controls Acta med scand Preprint
- III Lundbergh P & Strandell T : Hepatic wash-out curves of ⁸⁵Kr and ¹³³Xe after retrograde hepatic venous injections in patients with infectious hepatitis and control Accepted for publication in Scand J clin Lab Invest
- IV Lundbergh P : Hepatic circulation during and after infectious hepatitis Accepted for publication in Scand J Infect Dis

They will be referred to by the Roman numerals I-IV

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INTRODUCTION

About 2000 cases of viral hepatitis are reported in Sweden every year one-third of them being infectious hepatitis or viral hepatitis type A and two-thirds serum hepatitis or viral hepatitis type B. In accordance with WHO technical report (1973) the terms hepatitis A and hepatitis B will be used in this thesis.

Patients with viral hepatitis are anicteric or icteric and the disease is acute, subacute or chronic. The acute icteric form of hepatitis A is characterized by a pre-icteric stage with symptoms such as lassitude, loss of appetite, fever, nausea, vomiting, headache, abdominal discomfort, myalgia, arthralgia and exanthema. Within a week jaundice is noted. On physical examination the typical patient has a tender and enlarged liver but the hepatomegaly is rarely massive. The spleen is palpable in about 20 % of the patients (Sherlock 1968). The symptoms subside as jaundice fades and in adults the icteric stage lasts for an average of 3-4 weeks.

The histopathological changes seen at light microscopy of liver specimens from patients with acute hepatitis have been described by Robins & Iversen (1939) and by Locks (1944). It is usually believed that the picture is identical in hepatitis A and B but minor differences have been observed (Iwamoto et al 1972). The changes characteristic of both forms are single cell degeneration, focal necrosis, cellular infiltration of predominantly mononuclear cells, especially in the enlarged and edematous portal tracts and reticuloendothelial proliferation, foci with cells protruding into the hepatic sinusoids. Regeneration proceeds together with necrosis, the cells adjoining the central vein are usually the last to recover (Sherlock 1968) and on an average normal architecture is restored within 2-3 months (Wepl & Wildhirt 1968).

At electron microscopy the following hepatic changes have also been seen. The profiles of the rough endoplasmic reticulum are dilated (Jézéquel & Steiner 1967, Teodori et al 1967), sinusoidal microvilli are decreased in number and those present are often swollen (Teodori & Gentilini 1967); the hepatocytes are separated from each other (Ruebner & Slusser 1968); the space of Disse is widened and filled with amorphous debris and collagen fibrils (Teodori & Gentilini 1967); and basement membranes are present beneath the endothelial lining (Schaffner 1966).

During and after the second world war the importance of strict bed rest and high protein intake in the treatment of patients with viral hepatitis was stressed (Barker et al 1945 Hughes 1945). The importance of bed-rest in the healing of viral hepatitis was further emphasized by the findings in healthy subjects that hepatic blood flow decreased significantly on changes from supine to upright position (Culbertson et al 1947) as well as during exercise (Bradley 1949).

This strict bed rest regimen was soon questioned (Klatskin & Rappaport 1947 Gardner et al 1949 Swift et al 1950 Nelson et al 1954). The great challenge was made by Chalmers et al (1955) who in their thorough Korean study concluded: Patients hospitalized with acute infectious hepatitis improved as rapidly when allowed to be up and about their wards at will as did patients kept at strictly enforced bed rest. In a 10-year follow-up study by Nefzger & Chalmers (1963) no difference of significance was found between those treated with strict and those treated with ad libitum bed-rest. The opinion in the late 1960s as stated in current textbooks (Sherlock 1968 Moalloy & Galambos 1969) was that regardless of the depth of jaundice patients who had normal appetite and felt well may ambulate in the ward within the limits of physical fatigue.

To conclude patients with hepatitis A have symptoms and signs of impaired hepatic function that might be attributable to a reduced number of hepatocyte and an insufficiency of those surviving. The protrusion of cells into the sinusoids, changes of the sinusoidal surface widening of Disse's space and presence of basement membranes might affect local hepatic blood flow and the exchange between liver and blood thus contributing to the impaired liver function.

The object of the present investigation was to find out to what extent these morphologic changes of the liver in hepatitis A are attended with measurable changes in hepatic circulation by

- 1) analysing the hepatic circulation at rest and
- 2) studying the effect of exercise on hepatic circulation and finding out whether the chosen physical exercise provoked any signs of impaired liver function.

MATERIAL

Males between 20 and 30 years old with no history of drug or alcohol abuse treated for acute infectious hepatitis-B-antigen-(HBsAg)-negative viral hepatitis at the Roslagstull Hospital Stockholm over the period April 1972 - October 1973 were asked to participate in this study and 10 out of 14 patients all of whom consented. Although being sporadic the cases were diagnosed as hepatitis A since clinical and laboratory findings were not consistent with infection due to Epstein-Barr virus or to cytomegalovirus; the onset of the disease was acute and the patients were HBsAg-negative a few early (2-6) days after onset of jaundice (II). Therefore according to hospital routine liver biopsy was not performed. The results of the biochemical liver tests on the day of the catheterization are shown in Table I and their maximal abnormalities elsewhere (II). None of the patients had a previous history of liver disease and they were otherwise in good health. Patients nos 3, 6 and 7 were well-trained and the others ordinarily trained except patient no 10 who was untrained.

After clinical examination 17 healthy male volunteers of the same age were studied as a control group. None of them had previous history of liver, heart, lung or kidney disease and all denied drug and alcohol abuse. Routine urine and blood tests including standard biochemical liver test (Table I) showed no abnormalities and the subjects were all HBsAg-negative. Controls nos 4, 5 and 10 were well-trained the others ordinarily trained.

The total material consisted of 27 subjects (Table II). Three patients were re-examined but all the subjects did not participate in all the studies. The first study (I) comprised 10 controls nos 2, 3, 4, 15, 5, 16, 12, 17, 6 and 13; the second study (II) 10 patients nos 1, 10 and 13 controls nos 1-13; the third study (III) 8 patients nos 1, 2, 3, 5, 6, 7, 11 and 9 and 10 controls nos 1, 4, 5, 7, 8, 9, 12, 13, 14 and 15; and the fourth study (IV) 3 patients nos 7, 8 and 10.

There was no difference between the group of patients and the control group with respect to age, body size, blood volume, hemoglobin and hematocrit (Table II).

All subjects were studied in the morning without premedication. Because

of the risk of hypoglycemia in the patients during the 4-hour study including a 40-minute period of exercise the initial subjects were allowed a light breakfast 2-3 hours before the flow study. As there were no differences in blood-glucose values between patients and controls patients nos 7 8 9 and 10 and controls nos 8 9 10 and 11 were studied after overnight fast. By group comparison however no pertinent difference were found between fasting and non-fasting subject.

Table I Clinical and laboratory data on the day of the hepatic venous catheterization in 10 patients and corresponding group mean values for 13 controls. SBil = serum bilirubin ALP = alkaline phosphatase LDH = lactic dehydrogenase SGOT = serum glutamic oxaloacetic transaminase SGPT = serum glutamic pyruvic transaminase Thym = thymol turbidity U = units \bar{x} = group mean value SD = standard deviation xxx = $P < 0.001$ xx = $P < 0.01$ and x = $P < 0.05$ where P is the probability that the difference between patient and controls was caused by random factors. Upper normal limits given below each biochemical liver test.

	Day after onset of jaundice	SBil mg % <1.3	ALP U <3.0	LDH U <225	SGOT U <40	SGPT U <35	Thym U <6
Patient no							
1	15	1.6	2.6	152	43	146	9
2	23	1.8	2.4	184	81	206	11
3	14	5.8	4.0	199	320	970	6
4	12	1.8	2.6	145	120	213	2
5	14	1.4	2.5	126	36	150	2
6	10	9.6	2.7	339	710	740	13
7	22	3.5	2.9	132	79	350	4
8	15	1.1	3.5	170	78	360	14
9	17	2.6	2.5	134	37	100	12
10	16	2.1	2.6	141	88	270	-
\bar{x}	15.8	3.13	2.83	172.2	159.2	350.5	8.1
SD	4.0	2.66	0.52	63.3	210.4	284.1	4.7
Controls							
\bar{x}	-	0.87	1.84	159.7	18.5	16.9	1.4
SD	-	0.25	0.34	32.4	4.8	6.3	0.7
Difference	-	2.26	0.99	12.5	140.7	333.6	6.7
P	-	xxx ^{a)}	xxx	> 0.50	xxx ^{a)}	xxx	xxx

^{a)} Wilcoxon = non-parametric testing (Snedecor 1956)

Table II Anthropometric and laboratory data at rest (RI) in the patients and the controls
 BSA = body surface area WI = first work load WII = second work load Hb = hemoglobin Hct = hematocrit BV = blood volume PV = plasma volume RII = t 30 minutes after exercise
 Figure within parentheses are not included in mean value Other symbols as in Table I.

		Age yr	Height cm	Weight kg	BSA m ²	Work load kpm/min W1 W2		Duration min W3	Hb g% RI	Hct % RI	BV l RI	PV l RI	PV l RII
Patient no													
	1	29	177	67	1.85	500	600	21.00	11.7	36.1	4.30	2.89	2.86
	2	26	181	73	1.95	550	700	16.50	13.9	38.2	4.63	3.02	2.90
	3	27	182	67	1.89	250	500	20.75	13.9	40.8	5.76	3.63	3.26
	4	25	186	85	2.11	400	800	21.50	15.0	40.5	6.88	4.35	-
	5	21	180	62	1.82	400	700	16.25	14.7	41.1	4.62	2.89	2.74
	6	22	186	72	1.98	600	1000	15.25	14.6	43.7	5.63	3.39	-
	7	25	186	80	2.06	500	1000	20.75	10.9	34.0	6.65	4.59	4.38
	8	23	185	75	2.00	550	700	20.75	13.9	42.8	5.37	3.27	3.15
	9	25	190	83	2.13	550	700	20.75	12.7	39.2	4.76	3.06	3.93
	10	27	169	63	1.73	500	500	14.00	14.1	40.8	4.07	2.56	2.34
	\bar{X}	25.0	182	72.7	1.95	580	720	18.75	13.3	39.7	5.27	3.36	3.20
	SD	2.4	6	8.1	0.13	103	175	2.88	1.3	3.0	0.96	0.65	0.66
Control no													
	1	26	182	73	1.96	500	1000	21.00	14.2	36.0	4.24	3.26	-
	2	25	187	73	2.00	500	1000	17.70	15.5	42.1	5.13	3.17	-
	3	23	179	80	2.00	500	1000	13.75	15.6	40.6	5.56	3.51	-
	4	24	181	74	1.95	600	1200	21.25	14.4	40.0	6.54	4.16	-
	5	27	183	79	2.03	600	1200	21.35	14.9	40.0	5.21	3.31	2.90
	6	23	185	73	1.98	550	1000	10.00	15.8	47.1	7.00	4.00	3.70
	7	23	183	74	1.97	500	800	16.10	13.7	40.0	4.54	2.90	3.06
	8	25	177	64	1.82	550	700	21.70	14.3	43.5	4.30	2.60	2.40
	9	20	173	67	1.82	550	700	18.25	14.8	41.7	4.36	2.70	2.48
	10	29	192	92	2.25	400	1000	20.75	16.9	46.7	5.50	3.16	2.92
	11	26	185	74	1.99	500	1000	20.75	12.9	34.0	4.94	3.41	2.96
	12	24	187	73	2.00	600	1200	12.00	13.8	37.4	5.69	3.76	3.42
	13	27	175	72	1.88	500	1000	14.25	12.3	41.0	4.51	2.83	3.46
	14	(26)	(184)	(80)	(2.05)	(400)	(800)	(20.75)	(14.2)	(41.4)	(6.24)	(4.24)	-
	15	(21)	(185)	(83)	(2.08)	(450)	(900)	(21.40)	(16.1)	(43.2)	(5.05)	(3.07)	-
	16	(20)	(173)	(55)	(1.67)	(350)	(600)	(21.00)	(13.3)	(43.3)	(3.52)	(2.13)	-
	17	(26)	(193)	(100)	(2.34)	-	(1000)	(21.00)	(16.0)	(45.9)	(6.72)	(3.91)	-
	\bar{X}	24.8	182	74.5	1.97	495	985	17.60	14.5	40.8	5.24	3.27	3.03
	SD	2.3	5	6.7	0.11	85	168	4.02	1.3	3.7	0.82	0.50	0.44
Diff. P		0.2	0	-1.8	-0.02	-116	-265	1.15	1.2	-1.1	0.03	0.09	0.17
		>0.80	>0.99	>0.50	>0.60	xx	xx	>0.40	>0.05	>0.40	>0.90	>0.70	>0.50

METHODS

The subjects were studied during a resting period of about 1 hour during two consecutive 20-minute periods of supine leg exercise on an electro-dynamically braked bicycle ergometer (Holmgren & Mattsson 1954) and during 30 minutes after exercise. The second work load was generally twice as high as the first one and chosen so as to correspond to the heaviest load the subject was expected to sustain for 20 minutes. In 7 patients nos 1-7 and 4 controls nos 6, 7, 12 and 13 a 15-minute rest period was inserted between the two work loads in order to study the wedged and free hepatic venous pressure also after the first load.

In most subjects retrograde injections of ^{85}Kr and ^{133}Xe in saline were given into a cluded hepatic vein at rest before exercise to estimate the perfusion coefficients (III). No systematic influence of these injections on the hepatic extractions of oxygen and ICG was observed as judged from comparisons in patients nos 8 and 9 and controls nos 8 and 9 between blood sampled before the first injection and 2-4 minutes thereafter as well as before and after the subsequent injection.

The patients were investigated with utmost care special precautions being taken at all the procedures before, during and after the examination.

Catheterization. With the subject in the supine position a right hepatic vein was percutaneously catheterized from a cubital vein under TV fluoroscopy and the radiopaque teflon catheter (1 2/2 O.D. 1 5/2 3 mm) was placed as far caudally and peripherally as possible. In the wedged position the tip of the catheter was generally located 2-4 cm from the lateral chest wall and in the free position about 4 cm further proximal. Short teflon catheters (1 1/1 4 mm) were also percutaneously inserted into the brachial artery and a peripheral vein. In 2 patients nos 8 and 9 and 3 controls nos 7, 8, 9, 10 and 13 another right hepatic vein was similarly catheterized from the contralateral arm. In 3 patients nos 3, 5 and 6 and 4 controls nos 2, 3, 4 and 12 a thin polyethylene catheter (PE 60 O.D. 1 22 mm) was also percutaneously inserted from a cubital vein into the pulmonary artery.

Blood-flow measurements. After an i.v. bolus injection of about 15 mg of ICG (Cardio-Green® Hynson Westcott and Dunning Baltimore Md USA)

stabilized with 0.1% human albumin solution (Cherrick et al 1960) a constant i.v. infusion of the dye was given at a rate of about 1 mg/min. After an equilibration period of about 30 minutes 4-5 paired blood samples from the artery and hepatic vein were collected: the arterial sample about 15 seconds prior to the venous one. Blood was drawn at generally 5-minute intervals at rest during the work loads and the pause if any between them, and after exercise (Fig. 1). The plasma content of ICG was measured at 805 nm with individual calibration curves on a Hitachi 101 spectrophotometer (Hitachi Ltd Tokyo Japan) after correction for plasma turbidity at 900 nm (Nielsen 1963). The reproducibility was studied by

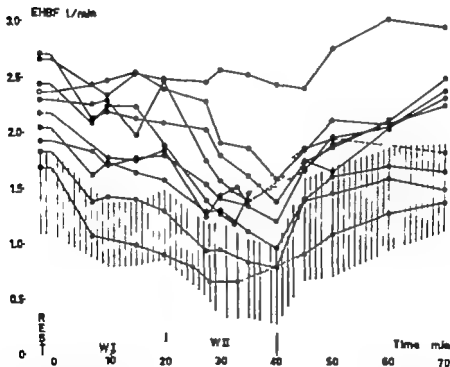


Fig. 1 Estimated hepatic blood flow (EHBF) at rest (open circles) during two supine 1 g bicycle work loads (WI and WII) and after exercise (filled circles) in 10 patients. Broken line indicates discontinued work load. Hatched area represents the range for 13 controls.
(From P. Lundbergh & T. Strömdell: Changes in hepatic circulation at rest during and after exercise in young males with infectious hepatitis compared with control. *Acta med scand*. In press.)

comparing dye concentration in blood sampled from 15 controls at 45 and 50 minutes after the start of the infusion. The coefficient of variation for a single determination thus including biological variation was 2.8 % for arterial ICG concentration and 5.1 % for arterio-hepatic venous dye difference with no systematic change with time. Generally a mean of 5 values from blood sampled between 30 and 50 minutes after the start of the infusion was used which reduced the error by a factor of 0.447.

Hematocrit (%) was determined in duplicates on all arterial samples by spinning the blood for 5 minutes at 9000 g in a microhematocrit centrifuge (MB International Equipment Co. Bedford, Mass. USA). The values were read under a magnifying glass. The reproducibility was studied by comparing mean values of blood sampled in 24 subjects at 45 and 50 minutes after the start of the ICG infusion. The coefficient of variation for a determination thus including biological variation was 0.7 % with no systematic change with time.

Plasma and blood volumes (1) were estimated at rest by i.v. injection of about 3 μ Ci 125 I human serum albumin. After an equilibration period of 10 minutes the activity of the indicator in blood was determined with a scintillator (Strandell & Lundbergh 1973) and the volumes were calculated with an assumed factor of 0.91 for total body hematocrit/large vessel hematocrit.

Total hepatic blood flow (l/min) at rest was calculated by the Bradley technique (Bradley et al. 1945) using, as recommended by Winkler et al. (1965) the average arterio-hepatic venous dye difference and the average change if any in arterial ICG concentration. The reproducibility was studied by comparing values of blood sampled from 25 subjects at 45 and 50 minutes after the start of the infusion. The coefficient of variation for a single determination thus including biological variation was 6.7 % with no systematic change with time. As earlier mentioned individual mean values of generally 5 ICG determinations were used, thereby reducing the error of the estimate by a factor of 0.447.

During and after exercise corrections for variations in arterial dye concentration due to change in plasma volume were performed mainly by

the method of Hultman & Castenfors (1961). The reproducibility was studied by comparing values of blood sampled from 22 subjects at 10 and 15 minutes after the start of the first work load. The coefficient of variation for a single determination thus including biological variation was 5.9% with no systematic change with time. The corresponding value at the second work load was 4.0% ($n=18$) with significant change with time.

Hepatic extraction of ICG (%) was measured as the arterio-hepatic venous difference divided by the arterial concentration.

Hemoglobin concentration (g/100 ml) and oxygen saturation (%) were calculated as mean values of 2-3 determinations on a CO-oximeter (IL 182 Lexington Mass. USA) calibrated against a standard of cyanmethemoglobin. The reproducibility of hemoglobin concentrations was studied by comparing mean values of arterial and hepatic venous blood sampled from 23 subjects at 45 and 50 minutes after the start of the ICG infusion. The coefficient of variation thus including biological variation was 1.0% with no systematic change with time. The corresponding coefficient of variation for a determination of hepatic venous oxygen saturation at rest was 1.8%. For duplicate determination on the same blood it was 0.3%. Oxygen saturation values determined on arterial blood on the CO-oximeter ($n=27$) were not significantly different ($+0.7\%$) from values obtained on a Beckman-8 spectrophotometer (Holmgren & Pernow 1959). Determinations on hepatic venous blood ($n=27$) with oxygen saturation values between 10 and 80% were however significantly higher averaging 1.8%. The arterio-hepatic venous oxygen difference calculated from values derived on the CO-oximeter was on an average 4% higher than when calculated from values determined on the Beckman-8 spectrophotometer; this discrepancy was not corrected for.

Splanchnic oxygen uptake (ml/min) was calculated as total hepatic blood flow multiplied by arterio-hepatic venous oxygen difference.

Total oxygen uptake and carbon dioxide elimination (l/min) were determined by the Douglas bag technique and micro-Schlander analysis. Expired air being collected during 8-10 minutes at rest generally during 2-3 minutes between the 16th and the 19th minute at the first load and during $\frac{1}{2}$ - 1 minute between the 17th and the 19th minute of the second load.

Cardiac output (l/min) was measured according to the direct Fick method by total oxygen uptake-determinations and calculations of arterio-venous oxygen difference from blood sampled from the pulmonary and brachial arteries

Heart rate (beats/min) was determined by electrocardiography

Intravascular pressures (mmHg) were measured by transducers (Bell & Howell no 1221 Woking Surrey England) using an ECGA-amplifier (SE labs Feltenham, Middlesex England) and a UV-writer (SE 3006 SE labs). Reference point for zero pressure was the midthoracic level of the sagittal chest diameter measured at the insertion of the fourth rib on the sternum. Free and wedged hepatic venous pressures were repeatedly recorded at rest before exercise during the pause if any between the loads and for 30 minutes after exercise. They were measured during quiet breathing and amplified so that 1 mmHg of pressure corresponded to a curve deflection of 1 cm. Individual mean values were calculated from which group means were derived. The undamped and damped curve was analysed to check that the catheter was properly wedged. For each measurement the pressure of the damped curve was integrated manually over 20-30 seconds and stated to the nearest 1/10 mmHg. The reproducibility was studied by comparing the last two pressure recordings during the initial rest. The coefficient of variation for a single determination was 6.0 % and 11.6 % (n=23) for wedged hepatic venous pressure and the difference between wedged and free hepatic venous pressure respectively. There was no systematic difference between the first and the second recording for any of these two variables. It was earlier (1) found that neither wedged hepatic venous pressure nor the difference between wedged and free hepatic venous pressure changed significantly after exercise during the 30-minute period studied. Therefore the individual mean values of all recorded pressures after exercise were used in further calculations.

Splanchnic flow resistance (mmHg/l/min) was derived as the fall in pressure (1 mean arterial blood pressure minus free hepatic venous pressure) divided by the total hepatic blood flow

Postsynovoidal flow resistance (mmHg/l/min) was derived as the fall in

pressure (i wedged minus free hepatic venous pressure) divided by the total hepatic blood flow

Clearance of ^{85}Kr and ^{133}Xe Local blood flows can be studied by measurement of the tissue clearance of ^{85}Kr and of ^{133}Xe (Kety 1951 Lassen & Munck 1955), as the diffusion of the isotopes is so rapid that at normal blood flows the exchange between tissue and blood is limited only by the capillary blood flow and not by diffusion barriers. The solubility in blood of krypton and of xenon depends mainly on the actual haematocrit (Hardewig et al 1960 Kitani 1972) and the solubility in liver mainly on the hepatic fat content (Andersen & Ladefoged 1967 Kitani & Winkler 1972). As krypton and xenon have markedly different solubility in fat difference in their partition coefficients between liver and blood have been used to calculate the hepatic triglyceride content (Kitani et al 1970).

^{85}Kr and ^{133}Xe (AB Atomenergi Studsvik Sweden) dissolved in saline in glass syringes were used as indicators. A small portion of the liver was labelled by slow retrograde hepatic venous injections of a 5-ml saline solution of the indicators with the hepatic venous catheter in the occluded position (Lassen 1965 Kitani et al 1970). Immediately after each injection which lasted for 5-10 seconds the catheter was withdrawn under TV fluoroscopy to a free position and flushed with 20 ml of saline. First one or two injections of about 1 mCi of ^{85}Kr were given then two or three injections of a mixture of about 1 mCi of ^{85}Kr and 0.02 mCi of ^{133}Xe . The time interval between two injections was 5-10 minutes.

The hepatic disappearance rate of the isotopes were simultaneously estimated from the count rate obtained from a 2 x 2 inch sodium iodide (Tl) scintillator connected to a two-channel pulse-height analyser and counter (Picker Digital Dual Rate Computer). The energy windows of the pulse-height analyser were set to record the peak gamma radiation from the two isotopes. The counts were accumulated for periods of 6 seconds and printed on paper. The first hepatic disappearance curve of ^{85}Kr alone were used to estimate the fraction of the krypton counts also recorded in the xenon window; this fraction was used to obtain net xenon activity. After semilogarithmic plotting the slope of the initial steepest exponential part of the disappearance curve was calculated as $\ln 2/t_{1/2}$ where $t_{1/2}$ denotes the time in minutes for the activity to decrease to half. The equations derived by

Kitani (1972) and Kitani & Winkler (1972) for calculations of the solubility in blood and in liver of the isotopes and others used for calculation of the partition coefficient between liver and blood for krypton and for xenon as well as for clearance and estimated hepatic fat content together with further details of the method, are given elsewhere (III)

The reproducibility was studied by comparing the half-times for the isotopes determined from the first and the second curve of simultaneously injected krypton and xenon. The coefficient of variation for a single determination of the half-time for krypton was 9.2 % and 7.2 % in patients and controls respectively. The corresponding values were 11.2 % and 9.5 % for xenon and 4.7 % and 4.9 % for the quotient between the half-time for krypton and for xenon. The coefficient of variation for a single determination of the partition coefficient was 9.7 % and 7.8 % for krypton in patients and controls respectively as against 14.3 % and 12.7 % for xenon. The corresponding values were 26.6 % and 24.9 % for estimated hepatic fat content. Individual mean values for half-times of the curves of krypton and xenon simultaneously injected were generally calculated which reduced these methodological errors by a factor of 0.707 or 0.577. There was no systematic difference between the first and the second determination for any of these variables.

Biochemical liver tests Serum bilirubin serum glutamic transaminase (SGOT) serum glutamic pyruvic transaminase (SGPT) serum lactic dehydrogenase (LDH) alkaline phosphatase and gamma-glutamyl transaminase (GGT) were determined at the Laboratory of Clinical Chemistry by standard methods on blood sampled at different intervals after exercise.

HBsAg testing Screening for HBsAg and performed at the Virus Department of the Stockholm County Council by a radioimmunoassay and immunoelectrophoresis (Berg

Statistical calculations were performed by the following probability (P) levels: $^{***}P < 0.001$ highly significant $^{**}P < 0.01$ probably significant Student's t-test

test were used as well as linear regression and correlation. Multiple regression analyses were performed according to the method of least square.

HEPATIC CIRCULATION AT REST

Previous investigations

In patients with viral hepatitis hepatic blood flow was first studied without hepatic venous catheterization by intravenous injection of various substances extracted from the blood by the liver and determination of their hepatic clearance. Normal (Krook 1956 Neumayr 1956) or decreased (Levy & Gliedman 1958 Burke & Gliedman 1959) clearance was observed with colloidal radiogold (^{198}Au) and normal clearance (Schmitt 1964) with colloidal chromium phosphate (^{32}P). Yet the hepatic extraction of the substance used is not complete and thus these methods give only the clearance although the data were interpreted as hepatic blood flow. The clearance is less than the hepatic blood flow however since $\text{clearance} = \text{flow} \times \text{extraction}$.

Normal values were calculated for hepatic blood flow both in the acute stage and after full convalescence after intrahepatic injections of radioactive iodinated serum albumin in 8 patients (Reichman et al 1957).

An adequate method for estimating total hepatic blood flow is by constant i.v. infusion of a dye such as bromsulphalein (Bradley et al 1945) or indocyanine green (Gassner et al 1961) hepatic venous catheterization and repeated measurement of the arterio-hepatic venous extraction of the dye at a constant arterial level. So far this method has been used in only one study in patients with viral hepatitis (Preisig et al 1966) yielding on an average normal hepatic blood flow but greater than normal splanchnic oxygen uptake.

Portal venous pressure has been studied indirectly in patients with viral hepatitis to find out whether or not this pressure would be affected by the disease. By wedging a catheter in a hepatic vein the pressure in the sinusoids is transmitted by the stagnant column of blood and can thus be measured. In the absence of presinusoidal block at which a normal wedged hepatic venous pressure can be found together with portal hypertension (Benhamou et al 1962) wedged hepatic venous pressure equals portal venous pressure. This similarity has been established in man at various pressure levels both during operation (Paton et al 1953 Cohn et al 1954 Welch et al 1954) and in awake subjects (Storti et al

1966 Viallat et al 1970) Paton et al (1953) recorded normal value for wedged hepatic venous pressure in 2 patients with acute hepatitis which indicated that the splenomegaly often attending hepatitis is not due to portal hypertension. Subsequent studies have also shown normal (Myhre 1954 Preisig et al 1966) or slightly increased (Balfour et al 1954 Krook 1956 Leavy & Gliedman 1958) values for wedged hepatic venous pressure in viral hepatitis.

Portal venous pressure as judged from intrasplenic pressure measurements in 40 cases of acute hepatitis was slightly but consistently elevated in the acute stage of the disease; the average decrease at re-examination after full convalescence was $5 \text{ cm H}_2\text{O}$ (Reichman & Davi 1957). No sustained portal hypertension was noted and none of the 21 patients studied by esophagoscopy had esophageal varices. However esophageal varices have been observed at esophagoscopy but not at X-ray examination in 35 of 82 patients with acute viral hepatitis (Harter & Palmer 1959). Portal venous pressure as measured by cannulation of the varices in some of these patients was also moderately elevated but there are no further report on esophageal varices in acute viral hepatitis.

A cordingly previous studies in patients with acute viral hepatitis have shown signs of normal or decreased total hepatic blood flows and normal or slightly increased portal venous pressure.

Present investigation

Table III summarizes the data for the group of patients and controls (Papers II-III) presented in Table IV-VI (Appendix) and in Table II (Paper III).

Estimated hepatic blood flow was significantly higher in the patients than in the controls (Tables III-IV). It was not related to any of the body-size variables. There was no relation of any significance in the patients between hepatic blood flow and the various biochemical liver tests, as a measure of the severity of the disease or the duration of jaundice.

Tabl. III Hepatic circulatory data and f t content in the group of patient and in the control group R = range and λ = partition coefficient between liver and blood Other symbols as in Tabl. I

		Patient	Controls	Difference	#
Estimated hepatic blood flow l/min	\bar{X} SD R	2.21 0.34 1.69-2.71	1.44 0.21 1.08-1.82	+0.77 +53%	xxx
Oxygen saturation in arterial blood, %	\bar{X} SD R	98.5 1.6 95.2-101.0	97.7 2.1 93.6-101.6	+0.8 +1%	>0.40
Oxygen saturation in hepatic venous blood %	\bar{X} SD R	79.2 2.9 75.5-86.3	76.4 5.0 69.4-84.9	+2.8 +4%	>0.10
Arterio-hepatic venous oxygen difference ml/l	\bar{X} SD R	36.1 4.8 27.6-44.9	43.0 7.8 32.0-56.2	-6.9 16%	x
Splanchnic oxygen uptake ml/min	\bar{X} SD R	79.7 16.0 60.9-109.4	60.9 9.2 48.8-74.4	+18.8 +31%	xx
Hepatic extraction f IO ₂ %	\bar{X} SD R	54.2 13.6 26.8-74.7	75.3 7 64.6-88.9	-21.1 -28%	xxx
Wedge hepatic venous pressure mmHg	\bar{X} SD R	10.1 2.2 8.3-14.2	8.7 1.7 5.6-11.8	+1.4 +16%	>0.10
Difference between wedge and free hepatic venous pressure mmHg	\bar{X} SD R	2.9 1.1 1.9-5.3	0.9 0.5 0.3-1.6	+2.0 +220%	xxx
Splanchnic flow resistance mmHg/l/min	\bar{X} SD R	37.5 8.9 29.1-54.1	60.0 9.7 48.7-82.9	-22.5 -38%	xxx
Portal sinusoidal flow resistance mmHg/l/min	\bar{X} SD R	1.3 0.5 0.7-2.2	0.7 0.4 0.2-1.4	+0.6 +86%	xx
λ Kr	\bar{X} SD R	1.25 0.14 1.04-1.46	1.09 0.06 1.00-1.21	+0.16 +15%	xx
λ I ₂	\bar{X} SD R	1.41 0.25 1.04-1.76	1.13 0.10 0.97-1.34	+0.28 +25%	xx
Estimated hepatic fat content % wet live weight	\bar{X} SD R	5.8 2.0 3.1-9.1	3.6 0.8 2.3-5.1	+2.2 +61%	xx
Clearance Kr ml/g/min	\bar{X} SD R	2.09 0.66 1.51-3.41	1.49 0.41 0.89-2.04	+0.60 +40%	x

Oxygen saturation in arterial and in hepatic venous blood. There was no significant difference between patients and controls in oxygen saturation either in arterial blood (Table III) or in hepatic venous blood (Table III V).

Arterio-hepatic venous oxygen difference was slightly lower in the patients than in the controls (Table III VI). The patients with the lowest value for arterio-hepatic venous oxygen difference had the highest value for serum bilirubin ($r = -0.648^{XX}$; $n=10$) and for SGPT ($r = -0.671^{XX}$; $n=10$).

Splanchnic oxygen uptake was significantly higher in the patients than in the controls (Table III VII) and significantly correlated to the biochemical liver tests as measure of the severity of the disease. Of the 10 patients those with the highest value for splanchnic oxygen uptake had the highest value for serum bilirubin ($r = 0.814^{XX}$) for SGOT ($r = 0.838^{XX}$) and for SGPT ($r = 0.855^{XX}$); no relationship was noted with the duration of jaundice. Splanchnic oxygen uptake at rest as a fraction of total oxygen uptake was on an average 0.34 (range 0.30 - 0.37) in 3 patients and 0.25 (range 0.20 - 0.29) in 4 controls.

Hepatic extraction of ICG was decreased in all but 2 patients; the average value was significantly lower for the patients than for the controls (Tables III VII). The patients with the highest value for serum bilirubin had the lowest hepatic extraction of ICG ($r = -0.856^{XX}$; $n=10$).

Wedge hepatic venous pressure was similar in patients and controls (Tables III VIII) and there was no significant relationship between this pressure and serum bilirubin, SGOT, SGPT or the duration of jaundice in any of the groups.

Free hepatic venous pressure was also similar in both groups averaging 7.3 and 7.8 mmHg in patients and controls respectively.

Difference between wedge and free hepatic venous pressure was significantly higher in the patients than in the controls (Tables III VIII). The largest

pressure difference was noted in the 10 patients with the most abnormal values for serum bilirubin ($r = 0.890^{XXX}$) for SGOT ($r = 0.883^{XXX}$) and for SGPT ($r = 0.666^X$). There was no significant relationship between this pressure difference and total hepatic blood flow in the patients. However, the patients with the highest values for this pressure difference had also the highest values for splanchnic oxygen uptake ($r = 0.761^X$; $n=10$).

Splanchnic flow resistance was significantly lower in the patients than in the controls (Table III, IX). In the patients there was no significant relationship between splanchnic flow resistance and the various biochemical liver tests.

Post-sinusoidal flow resistance was significantly higher in the patients than in the controls (Table III). The patients with the highest values for post-sinusoidal flow resistance had the highest values for serum bilirubin ($r = 0.682^X$; $n=9$) and for arterio-hepatic venous oxygen difference ($r = 0.844^{XX}$; $n=9$).

Clearance of ^{85}Kr and ^{133}Xe . There was no significant difference between the half-time of the two groups, whereas the average quotient between the half-time for krypton and for xenon was significantly lower in the patients than in the controls (III). The partition coefficient between liver and blood for both isotopes was significantly higher in the group of patients than in the controls, as was the average estimated hepatic fat content: 5.8 % as against 3.6 % (Table III). The hepatic clearance of krypton was also higher in the patients than in the controls (Table III).

As regards the relationship between estimated hepatic fat content on one hand and biochemical and physiological hepatic variables on the other, the hepatic fat content was highest in the patients with the least abnormal findings. The patient with the highest hepatic fat content thus had the lowest values for serum bilirubin ($r = -0.734^X$; $n=8$) and for SGOT ($r = -0.696^X$; $n=8$) and the lowest values for the difference between wedged and free hepatic venous pressure ($r = -0.775^X$; $n=8$) and for post-sinusoidal flow resistance ($r = -0.682^X$; $n=7$). No corresponding relationships were observed in the group of patients between clearance of krypton and the other variables.

Re-examination of 3 patients 5 weeks after the first study showed that the values for all variable studied (IV) qualified the data in the earlier examined control group (II-III)

Discussion

The patients and the controls were studied by the same method. The data in the controls were in good agreement with results obtained by others (Bradley 1949 Wade et al 1956 Bishop et al 1957 Bradley 1963 Rowell et al 1964 Mahren et al 1971 Kitani & Winkler 1972) and data at re-examination of 3 patients (IV) when they had almost completely recovered from the hepatitis were similar to results in the earlier studied control group (II-III). Therefore the hepatic circulatory and lipid abnormalities observed in the patients during the acute stage of the disease seemed to be attributable to the disease and its treatment and not to selection. However the dietary and medical treatment of the patients cannot have affected the hepatic circulation significantly; only once or twice a week was the normal hospital diet replaced by a meal less rich in fat and the only medical treatment was vitamin pills twice the daily requirement.

The hyperkinetic hepatic circulation was not part of a general vasodilatation. In 3 patients cardiac output at rest was on an average 17 l/min and hepatic blood flow 2.6 l/min . The corresponding values for 4 controls were 7.3 l/min and 1.5 l/min . The average flow of blood to extrahepatic parts of the body was thus similar in both groups 6.1 l/min in the patient and 5.8 l/min in the controls.

The high hepatic blood flows in the patients with hepatitis are in agreement with the hyperemia in the diseased organs in various infections. In previous reports however total hepatic blood flow has been estimated to be normal (Krook 1956 Neumayr 1956 Reichman et al 1957 Schmitt 1964 Preisig et al 1966) or decreased (Levy & Gliedman 1958 Burke & Gliedman 1959). Since all these authors except Reichman et al (1957) and Preisig et al (1966) used the clearance method their values do not reflect hepatic blood flow. Preisig et al (1966) in 14 patients with serum bilirubin between 2.4 and 32 mg/100 ml studied within an average

of 19 days of the onset of hepatitis found total hepatic blood flow to be normal namely 1510 (890 - 2430) ml/min. The discrepancy between their data and the present findings cannot be explained.

It has been shown in man that total hepatic blood flow can increase markedly after typhoid vaccination even when fever was prevented (Bradley 1949). In rabbit an increased hepatic clearance of ICG was observed for many weeks after i.v. injection of bacterial gous protein (Lang et al. 1965) which was considered to be due to an induced stimulation of the parenchymal cells maybe by a higher blood flow. The present findings do not allow any conclusions as to whether or not immunologic factors contribute to the observed high hepatic blood flows in acute viral hepatitis.

A more probable cause of the high hepatic blood flow might be the unevenly distributed hepatic cellular changes also impairing local circulation unevenly leading to anoxia in some areas. A resultant metabolic vasodilatation might correspond to the most anoxic regions in the liver rather than to the average requirement of oxygen. Thereby total hepatic blood flow would be increased in excess of metabolic demand resulting in a lower arterio-venous oxygen difference and a higher oxygen saturation in the hepatic venous blood as in the present study. Such a local hepatic vasodilatation cannot be mediated by the portal vein since a decrease in the small portal venous flow resistance over the liver is unlikely and anyhow cannot measurably increase the portal venous blood flow. On the contrary an increased flow resistance over the portal sinusoids should be expected as in the sinusoidal type of portal hypertension (Rappaport et al. 1970) and such an increased postsinusoidal flow resistance was also observed in the present study probably caused by swelling of the hepatocytes and/or changed microcirculation with an increased hepatic artery blood flow. The changes seen at light microscopy of liver specimens from patient with viral hepatitis are similar irrespective of the type of the virus. The observed change in hepatic circulation in the present patient with hepatitis A might also be expected in patients with other forms of hepatitis caused by viruses.

In 1 patient the wedged hepatic venous pressure was recorded after exercise showing a marked wave resembling an arterial curve and a pressure

1 val close to the arterial one. In some other patient unstable wedged pressure recordings were obtained after exercise showing sliding pressures up to 30-40 mmHg which cannot represent portal venous pressure. An altered hepatic microcirculation with increased hepatic artery blood flow might explain these findings. Although the absolute value of the difference between the patients and the controls was low as regards the difference between wedged and free hepatic venous pressure it corresponded to twice as high a flow resistance in the patients as in the control. Despite these significant changes the wedged hepatic venous pressure was not significantly higher in the patients than in the controls in agreement with the findings of others (Paton et al. 1953, Balfour et al. 1954, Myhre 1954, Preisig et al. 1966). Previously reported signs of increased portal venous pressure (Krook 1956, Reichman & Davi 1957, Leavy & Gliedman 1958, Haerte & Palmer 1959) probably indicate only somewhat more marked hepatic changes than in the present study.

The indication of higher than normal oxygen consumption during regeneration of hepatic tissue (Brauer 1963) might explain the present increase in splanchnic oxygen uptake in agreement with the findings of Preisig et al. (1966). In the present patients the values of the biochemical liver test as measures of the degree of hepatic abnormality were more closely correlated to the splanchnic oxygen uptake than to the total hepatic blood flow probably owing to the fact that total hepatic blood flow was more influenced by other factors such as the sympathetic activity at the time of the study.

The validity of the observed clearance of krypton as a measure of the average perfusion coefficient was tested by dividing the individual value of total hepatic blood flow estimated by the Bradley technique (II) by the krypton clearance. The "liver weight" thus calculated were significantly lower than the expected value of 1200 - 1600 g in normals (Sheelock 1968, Rappaport 1969) the average value being 1045 (range 700 - 1520) g in the controls and 1145 (range 690 - 1615) g in the patient. The clearance of krypton thus overestimated the hepatic perfusion coefficient in the control by an average of 25 % possibly because of local hyperemia in the injected area similar to what has been observed after injection into skeletal muscle (Tønnesen & Sejrsen 1970). The previously reported range of hepatic clearance of krypton

(Kitani et al 1970) was of the same magnitude as that in the present control but lower than that in the patients.

The amount of hepatic fat can be estimated in vivo in different ways. At microscopy of liver specimen no lipid is generally seen unless a high percentage of fat is present (Billing et al 1953). The fat content can be determined chemically after liver biopsy (Kramer et al 1969, Laurell & Lundquist 1971) but in the individual case the representativity of this small specimen may be questioned. During a hepatic blood flow study and without liver biopsy (III) it is possible to estimate the hepatic triglyceride content (Kitani & Winkler 1972). This seems to be appropriate although other lipids such as cholesterol and phospholipid with different solubility for the isotopes are also present in the liver since the greatest variation in hepatic fat content in healthy subjects and especially in patients with liver disorders is caused by triglyceride (Klenk 1964, Kramer et al 1969).

An increased hepatic fat content has been observed in viral hepatitis both in the acute stage (Christoffersen 1973) and during restitution (Colwell 1954). In the present series the hepatic fat content was highest in the patient with the less marked abnormalities of the biochemical liver tests and of the circulatory data (III) probably because these patients were studied in a later stage of the disease. The hepatic fat content was normal at re-examination of the 2 subjects (IV) who had had the highest values (III). This temporary increase in hepatic fat content might depend on dietary factors (Colwell 1954, Laurell & Lundquist 1971). Another explanation could be a temporary metabolic insufficiency of the regenerating hepatocytes.

As no major change in central circulation was observed in the acute stage of hepatitis A (II) it seems very unlikely indeed that a short period of bed rest and libitum could in itself cause increases of hepatic blood flow, splanchnic oxygen uptake and hepatic fat content. Thus the changes observed in the present cases during hepatitis A should be related to the disease. Judged from these few patients in the present study the hepatic circulatory and lipid abnormalities during acute stage of uncomplicated hepatitis might be expected to disappear within 2 months of the onset of symptoms. This is in good agreement with the finding that the histological changes in viral hepatitis are usually restituted 2-3 months after onset of the disease (Wepler & Wildhirt 1968).

HEPATIC CIRCULATION DURING AND AFTER EXERCISE

Previous investigation

When healthy subjects perform physical exercise total hepatic blood flow is reduced (Bradley 1949) the heavier the exercise the greater the flow reduction (Made et al 1956 Bishop et al 1957) During maximal work in the upright position total hepatic blood flow has been found to be only 20 % of the value at rest (Rowell et al 1964) This decrease in hepatic blood flow during exercise has been found to be more closely related to the relative than to the absolute work load of the individual (Rowell et al 1964)

In patients with viral hepatitis the effect of exercise on hepatic blood flow has not been studied directly So far the only effects that have been studied are those on recovery and relapse rate Swift et al (1950) and Krikler & Zilberg (1966) report negative effects on the liver of exercise performed in the acute stage of the disease Repsher & Freebern (1969) and Edlund (1971) on the other hand found no undue effects on the liver after strenuous physical exercise Swift et al (1950) noticed that the mean duration of the disease was about 2 weeks longer after exercise in patients with serum bilirubin value above 3 mg/100 ml than in those with values lower than 3 mg/100 ml Krikler & Zilberg (1966) noted deleterious effects of physical exercise in that 3 of their 5 patients who performed strenuous exercise in the pre-icteric stage of the hepatitis died within 4-16 days of the onset of the disease but give no data on the numbers of patients with hepatitis who perform physical exercise during the pre-icteric period of the disease without negative effects on recovery and relapse rate Repsher & Freebern (1969) investigated 396 American servicemen with hepatitis A in Vietnam; all of them had increased level of serum bilirubin and SOOT onset of symptoms less than 14 days before admission and an asymptomatic period of less than 5 days One-half of the men were ambulant in the ward without prescribed exercise The other half had daily exercise for 3 hours a week their symptoms were considered to be slight irrespective of the degree of the abnormalities in their biochemical liver test ; average serum bilirubin was 7.5 mg/100 ml No adverse effects of the exercise were noted on recovery or relapse rate However neither the material nor the intensity of the exercise was clearly defined Edlund (1971) found

no delayed recovery and no deleterious effects on the liver in 23 patients with acute viral hepatitis who when SGPT was below 300 unit performed a moderately heavy bicycle exercise for 12 minutes on 6 consecutive days

Present investigation

The 10 patients and 13 controls were studied during two 20-minute periods of supine leg exercise and for 30 minutes thereafter (I II). The average heart rate was 126 beats/min and 116 beats/min in patients and controls respectively at the end of the first work load averaging 380 and 496 kpm/min (Table II X). At the end of the second work load the average heart rate was 166 beats/min and 175 beats/min for patients and controls respectively at 720 and 985 kpm/min (Tables II X). The difference in heart rate between the groups was not significant at any of the loads. The first 4 patients studied had heart rates not exceeding 160 beats/min as had also 1 control. In relation to work load the mean heart rate of the patients was thus about 15 beats/min higher than that of the control (Fig. 2).

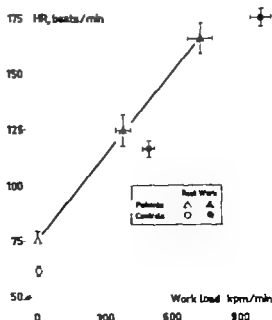


Fig. 2 Heart rate (HR) at rest (per symbols) and at the end of two generally 20-minute periods of supine leg exercise (filled symbols) in relation to work load in 10 patients (triangles and solid line) and in 13 controls (circles and broken lines). Group mean and standard error of the mean are given.

Hepatic blood flow During the first work load total hepatic blood flow decreased by an average of 15-20 % in both groups (Fig 1 Table IV); the difference in absolute flow value between the groups was highly significant. Even lower total hepatic blood flows were observed in all patients but one no 3 during the second load averaging 1.30 l/min and 0.56 l/min respectively in patients and controls (Table IV) or 59 % and 39 % of the respective value at rest. The hepatic blood flow for both groups is shown in Fig 3 in relation to absolute work load and in Fig 4 to heart rate as a measure of the relative work load. When the reduced hepatic blood flow during exercise was expressed in per cent of the value at rest the patient and the controls had the same flow reduction in relation to the increase in heart rate (Fig 5) as a measure of the relative work load. The increase in splanchnic flow resistance with exercise was not significantly different between patients and controls when related to the simultaneous increase in heart rate.

At 30 minutes after exercise the values for total hepatic blood flow (Table IV) mean arterial blood pressure (Table IX) and splanchnic flow resistance (Table IX) had returned to the initial value at rest in both groups but the heart rates (Table X) were still significantly elevated above initial resting value.

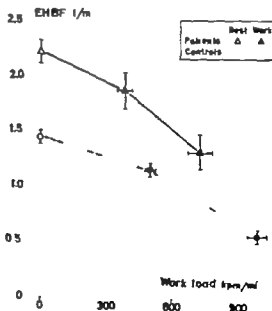


Fig. 3 Estimated hepatic blood flow (EHBF) at rest and at the end of two generally 20-minute periods of supine leg exercise in relation to work load in 10 patients and 13 controls. Group mean and standard errors of the mean are given. Symbol as in Fig. 2.

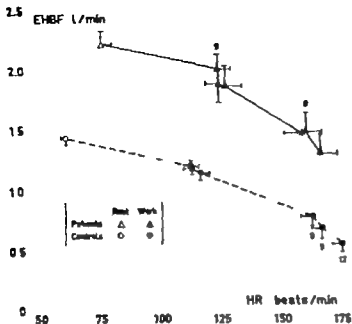


Fig. 4 Estimated hepatic blood flow (EHBF) at rest after 10 and 15 minutes and at the end of two generally 20-minute periods of supine leg exercise in relation to heart rate (HR) in 10 patients and 13 controls. Group means and standard errors of the mean are given. No. of individuals is set out when reduced. Symbols as in Fig. 2. (Reproduced from Paper II)

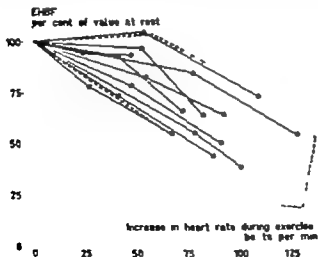


Fig. 5 Change in estimated hepatic blood flow (EHBF) during exercise (end of two generally 20-minute periods of supine leg exercise) in relation to change in heart rate in 10 patients. Area within broken line represents range for 13 controls. (Reproduced from Paper II)

Hepatic extraction In the patients the average hepatic venous oxygen saturation was reduced from 79 % at rest to 74 % and 60 % at the end of the first and second work loads respectively. The corresponding values for the controls were 76 % at rest and 70 % and 35 % during exercise (Table V). Oxygen saturation in arterial blood did not differ between the groups at any of the loads and was not significantly different from the values at rest. The resultant arterio-hepatic venous oxygen difference was lower in the patients than in the controls during both loads (Fig. 6 Table VI). Splanchnic oxygen uptake did not change significantly in any of the groups during exercise compared with that at rest (Table VII). Accordingly the increase in arterio-hepatic venous oxygen difference during exercise balanced the decrease in total hepatic blood flow.

At 30 minutes after exercise hepatic venous oxygen saturation (Table V) and arterio-hepatic venous oxygen difference (Table VI) were still significantly changed compared with the values at rest in the group of patients but not in the group of controls.

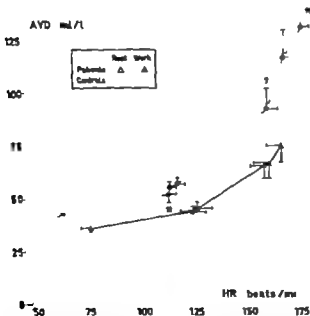


Fig. 6 Arterio-hepatic venous oxygen difference (AVD) at rest after 10 and 15 minutes and at the end of two generally 20-minute periods of supine leg exercise in relation to heart rate (HR) in 10 patients and 12 controls. Group means and standard errors of the mean are given. No of individual is set out when reduced. Symbols as in Fig. 2. (Reproduced from Paper II)

Hepatic venous pressures. In both groups wedged and especially free hepatic venous pressures were lower when measured in the 30-minute period after exercise (Table VIII). During the 15-minute pause between the loads the average wedged hepatic venous pressure was unchanged compared with the value before exercise in patients and controls thus studied. In both groups the average difference between wedged and free hepatic venous pressure increased after exercise but this change was significant for the controls alone. After the first load the average pressure difference was virtually unchanged compared with that at rest 3.4 mmHg and 2.9 mmHg respectively in the patient and 1.1 mmHg and 0.9 mmHg in the controls.

Effect of exercise on symptoms and biochemical liver test. In the 8 patients who at the time of the study had serum bilirubin values below 4 mg/100 ml and SGOT and SGPT below 150 units and 400 units respectively no negative effects of the exercise were noted on recovery or relapse rate (II). Of the other 2 patients no. 3 showed a normal recovery and the values of his biochemical liver tests decreased except for a slight and temporary increase of SGOT from 300 to 390 units between the 4th and the 6th day. Thereafter SGOT decreased normally and the recovery was uneventful. This patient was the only one in whom no decrease of hepatic blood flow was noted during exercise and in whom a flow increase above the resting value was recorded after exercise. In patient no. 6 the symptoms subsided and on the 4th day serum bilirubin was 8.4 mg/100 ml whereas SGOT and SGPT both were 1000 units. On the 6th day the corresponding values were 7.1, 960 and 1000. Ten days after the catheterisation the patient experienced malaise, fatigue, loss of appetite and increased jaundice. By the 11th day serum bilirubin had risen to 11.3 mg/100 ml whereas SGOT and SGPT had decreased to 700 units and 840 units respectively. One week later he began to improve clinically in spite of a serum bilirubin value of 13.1 mg/100 ml; SGOT and SGPT were down to 460 units and 490 units respectively. Thereafter serum bilirubin decreased and the recovery was uneventful and the patient was discharged 5 weeks after catheterisation.

Discussion

The patients and the controls were studied at similar heart rates the values for the patients being somewhat lower than those for the controls mostly owing to the patient's lower working capacity (Fig. 2) but also to the fact that the first 4 patients were studied at a capacity lower than maximal. Since the pertinent comparison during exercise between patient and controls were made in relation to heart rate as a measure of relative work load (Sjödstrand 1960) the present differences in work load between patients and controls are of minor importance. The average difference in heart rate in relation to work load of about 20 beats/min between the acute stage and convalescence in the 3 patients who were re-examined (IV) was similar to the difference between the 10 patients and the 13 controls (II). Therefore the higher heart rate during exercise of the patients compared with those of the controls were probably caused by the disease or its treatment by bed-rest rather than by selection or by difference in premorbid physical capacity.

In spite of great differences in flow values between patients and controls and signs of increased hepatic artery blood flow in the patients the decrease in total hepatic blood flow during exercise and the increase in splanchnic flow resistance were equal in both groups in relation to the increase in heart rate indicating the same sympathetic flow control in both groups (Fig. 5). There were no signs of the splanchnic oxygen uptake being affected in the patients by the decrease in hepatic blood flow during exercise.

It has been suggested that the increase after exercise in the difference between wedged and free hepatic venous pressure might be related to the increased postsinusoidal flow resistance due to swelling of the hepatocyte possibly evoked by ischemia during the exercise (I). In the present patients with hepatitis no such increase in pressure difference was noted after exercise. The 2 patients nos. 3 and 6 who had the highest values of serum bilirubin at the time of the study had also the highest difference between wedged and free hepatic venous pressure but only an average increase of this pressure difference after exercise. In one of these patients no. 3 an increase of hepatic blood flow above the resting value was noted after exercise which might be interpreted as a sign of post-exercise metabolic vasodilatation. Thus the present study does not favour the view that the

increase in postsinusoidal flow resistance after exercise is related to hepatic ischemia but seems to show that the increase is related to altered hepatic microcirculation due to other causes

None of the present 8 patients with values for serum bilirubin below 4 mg/100 ml and for SGOT and SGPT below 150 units and 400 units respectively at the time of the catheterization showed any symptoms or signs of negative effects of the exercise. Of the 2 patients nos 3 and 6, with serum bilirubin values of 5.8 mg/100 ml and 9.6 mg/100 ml respectively no 3 showed a slight delay in the decrease of SGOT and no 6 a relapse that delayed his recovery for about 2 weeks. Patient no 3 performed only 500 kpm/min with a final heart rate of 120 beats/min whereas patient no 6 was well-trained and performed a heaviest load of 1000 kpm/min with a final heart rate of 180 beats/min. This prolonged recovery may have been due to chance since according to current textbooks (Sherlock 1968 Mosley & Galambos 1969) and the experience in this hospital this clinical course occurs in about 10 % of patients with acute viral hepatitis. However if they are not due to chance these increases in values for biochemical live tests after exercise in 2 patients show that signs of liver cell damage can be provoked during exercise even if total hepatic blood flow is kept within normal resting limits. This might be explained by heterogeneity of hepatic blood flow and local areas of ischemia. Thus the possibility that the physical exercise in these 2 patient may have been the provoking factor cannot be excluded and pending more extensive studies heavy physical exercise in hepatitis A should be avoided until serum bilirubin is less than about 4 mg/100 ml. This supports Wift et al (1950) conclusion that in patients with serum bilirubin values lower than 3 mg/100 ml exercise did not prolong the duration of the disease.

GENERAL SUMMARY

The study comprised 10 male patients and 17 healthy male volunteers as controls. All were between 20 and 30 years old with similar degree of physical training; none had a history of drug or alcohol abuse and all were hepatitis-B-antigen (HBsAg)-negative. The patients were treated for acute icteric viral hepatitis at the Roslagstull Hospital, Stockholm, over the period April 1972 - October 1973. Although being sporadic the cases were diagnosed as hepatitis A since clinical and laboratory findings were not consistent with infection due to Epstein-Barr virus or cytomegalovirus; the onset of disease was acute and the patients were HBsAg-negative as early as 5 (2-8) days after onset of jaundice.

Hepatic circulation was studied in the supine position by hepatic venous catheterization at rest and during two generally 20-minute periods of supine light exercise and for 30 minutes thereafter. The second work load was twice as high as the first one and chosen so as to correspond to the heaviest load the subject was expected to sustain for another 20 minutes. Total hepatic blood flow was estimated according to the Bradley technique with intravenous constant infusion of indocyanine green dye. Arterio-hepatic venous oxygen difference was determined and splanchnic oxygen uptake calculated. Wedged and free hepatic venous pressures and the difference between them were measured and splanchnic flow resistance and post-sinusoidal flow resistance calculated. In 8 patients and 10 control injections of a solution of ^{85}Kr and ^{133}Xe in saline were also given at rest into an occluded hepatic vein and the disappearance rates of the isotopes were measured with an external scintillator over the liver and given as the half-times of the initial steepest exponential part of the disappearance curve. The triglyceride part of the hepatic fat content was estimated from actual hematocrit and the quotient between the half-time for krypton and for xenon. Partition coefficients between liver and blood were calculated as well as clearance of krypton.

At rest in the supine position the patients had significantly higher values than the controls for total hepatic blood flow (2.21 l/min as against 1.44 l/min) for splanchnic oxygen uptake (79.7 ml/min versus 60.9 ml/min) for the difference between wedged and free hepatic venous pressure (2.9 mmHg as against 0.9 mmHg) and for estimated hepatic fat content (5.8 % versus 3.6 %). Arterio-hepatic venous oxygen difference, splanchnic flow resistance,

and hepatic extraction of ICG were lower whereas the clearance of krypton was higher in the group of patients than in the control group

At re-examination of 3 patients 5 weeks after the first study all values had decreased markedly as compared with the first study hepatic blood flow and splanchnic oxygen uptake by 20-25 % and all values equalled those of the control group

During supine leg exercise the 10 patients and 13 controls were studied at final heart rates averaging 166 beats/min and 175 beats/min respectively at the heavy st work load of 720 kpm/min and 985 kpm/min The difference in heart rate was not significant but in relation to work load the patients had about 15 beats/min higher heart rates than the controls During exercise total hepatic blood flow decreased in both groups by about 0.8 l/min When the reduced hepatic blood flow during exercise was expressed in per cent of the value at rest the patients and the controls had the same flow reduction and increase in splanchnic flow resistance in relation to the increase in heart rate as a measure of the relative work load Arterio-hepatic venous oxygen difference during exercise was lower in patients than in controls The increase with exercise balanced the decrease in hepatic blood flow indicating an unchanged splanchnic oxygen uptake during exercise

After exercise wedged hepatic venous pressure was lower in both groups but since free hepatic venous pressure was still lower the difference between wedged and free hepatic venous pressure was higher in both groups significantly so in the controls At 30 minutes after exercise the values for total hepatic blood flow and splanchnic flow resistance had returned to the initial values at rest in both groups Arterio-hepatic venous oxygen difference and heart rates were however still significantly higher than the initial resting values in the group of patients

No negative effects of the exercise were noted in the 8 patients with values for serum bilirubin below 4 mg/100 ml whereas a temporary increase and delayed decrease of both serum transaminases and serum bilirubin were noted in the remaining 2 patients with serum bilirubin values of 9.6 mg/100 ml and 5.8 mg/100 ml respectively

A probable explanation of the high hepatic blood flow seen in the acute stage of hepatitis A in the present study might be that because of the

uneven distribution of the hepatic cellular changes local circulation would be unevenly impaired and anoxia thus develop in some areas with a resultant metabolic vasodilatation. The increase in regeneration of liver cells might have contributed as both total hepatic blood flow and splanchnic oxygen uptake were elevated. There were no signs indicating that the splanchnic oxygen uptake was affected by the decrease of hepatic blood flow during exercise. The increase of hepatic fat content might depend on dietary factor and/or a temporary metabolic insufficiency of the regenerating hepatocytes.

The fact that the decrease in hepatic blood flow and the increase in splanchnic flow resistance with exercise were similar in patients and controls indicated the same sympathetic flow control during exercise in both groups.

It was concluded that the observed changes in the acute stage of hepatitis were attributable to the disease and that they were reversible. Pending more extensive studies strenuous physical exercise should be avoided in patients with hepatitis A in the acute stage until serum bilirubin is below 4 mg/100 ml.

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REFERENCES

- Andersen A M & Ladefoged J : Partition coefficient of ¹³³xenon between various tissues and blood in vivo Scand J clin Lab Invest 19:72 1967
- Balfour Jr D C Reynolds T B Mikkelsen W P Pattison A C & Hales M R : Portal hypertension Arch intern Med 94:853 1954
- Barker M H Cappe H B & Allen F W : Acute infectious hepatitis in the Mediterranean theater JAMA 128 997 1945
- Benhamou J -P Girond C Guillemot R Tricot R & Pauvert R : Pre-sinusoidal block in cirrhosis of the liver in Martini G A (ed) Aktuelle Probleme der Hepatologi pp 74-78 G Thieme Verlag Stuttgart 1962
- Berg R Berntsen K O Björling H Holmström E & Vyas G N : Recovery of hepatitis B antibody from human plasma products separated by a modified Cohn fractionation Vox Sang 1974 In press
- Berg R Ringert O & Espmark Å Australia antigen in hepatitis among Swedish track finders Acta pathol microbiol scand Section B 79:423 1971
- Billing B H Conlon R J Hein D E & Schiff L : The value of needle biopsy in the chemical stimulation of liver lipids in man J clin Invest 32 214 1953
- Bishop J M Donald K W Taylor S E & Wornald P W Changes in arterial-hepatic venous oxygen content difference during and after supine 1 g exercise J Physiol (Lond) 137:309 1957
- Bradley S E Variations in hepatic blood flow in man during health and disease New Engl J Med 240:456 1949
- Bradley S E The hepatic circulation in Hamilton W F (ed) Handbook of physiology Section 2: Circulation volume II pp 1387-1438 American Physiological Society Washington D C 1963
- Bradley S E Ingelfinger F J Bradley G P & Curry J J The estimation of hepatic blood flow in man J clin Invest 24 890 1945

- Brauer R.W. Liver circulation and function *Physiol Rev* 43 115
1963
- Burkle J S & Gliedman M.L.: External recording method for estimating hepatic blood flow with the use of radiogold *Gastroenterology* 36 112
1959
- Caesar J Shaldon S Chiandussi L. Quevara L. & Sherlock S
The use of indocyanine green in the measurement of hepatic blood flow
and as a test of hepatic function *Clin Sci* 21 43 1961
- Chalmers T C Eckhardt R D Reynolds W E Cigarroa Jr J O
Deane W Reifenshtein R W Smith C W & Davidson C S The
treatment of acute infectious hepatitis: Controlled study of the
effect of diet, rest and physical reconditioning on the acute
course of the disease and on the incidence of relapses and residual
abnormalities *J Clin Invest* 34 1163 1955
- Cherrick G R Stein S W Leevy C M. & Davidson C S Indocyanine
green Observations on its physical properties plasma decay and
hepatic extraction *J Clin Invest* 39 592 1960
- Christofferson P Personal communication 1973
- Cohn R Ordway G & Ellis E Relation of portal venous pressure to
labeled hepatic venous pressure *Arch Surg* 69 853 1954
- Cobwell Jr A R. Occurrence of accumulation of fat in the liver and
its relation to excess weight gain in patients convalescing from viral
hepatitis *Ann Intern Med* 41 963 1954
- Culbertson J W Wilkins R W Ingelfinger F J & Bradley H E
The effect of the upright posture upon hepatic blood flow in normal
and hypertensive human subjects *J Clin Invest* 26 1178 1947
- Edlund A. The effect of defined physical exercise in the early
convalescence of viral hepatitis *Scand J Infect Dis* 3 189 1971
- Gardner H T Roelstad R A Moore D J Streitfeld F A &
Knowlton M Hepatitis among American occupation troops in Germany:
A follow-up study with particular reference to interim alcohol and

physical activity Ann Intern Med 30:1009 1949

Raeter W & Palmer E D : Portal hypertension with esophageal varices in acute infectious hepatitis: Further observations Amer J Med Sci 237:1596 1959

Hardewig A Rochester D F & Briscoe W A : Measurement of solubility coefficients of krypton in water plasma and human blood using radioactive Kr⁸⁵ J appl Physiol 15:723 1960

Holmgren A & Mattsson E -H A new ergometer with constant work load at varying pedalling rate Scand J clin Lab Invest 6:137 1954

Holmgren A & Pernow B : Spectrophotometric measurement of oxygen saturation of blood in the determination of cardiac output A comparison with the Van Slyk method Scand J clin Lab Invest 11:143 1959

Hughes J D The treatment of infectious hepatitis by diet and rest Bull U S Army med Dep 4 662 1945

Hultman E & Castenfeldt H Effect of injection of hypertonic glucose on splanchnic blood flow and oxygen consumption Scand J clin Lab Invest 13:103 1961

Ivarson S Lundin B & Hernodsson S Liver morphology in acute viral hepatitis related to the hepatitis B antigen J clin Path 25 850 1972

Jéséquel A M & teiner J W Electron microscopy of liver biopsies and of transmissible agents from hepatitis patient T Gastro-Ent 106: 3 1967 (suppl)

Kety S The theory and applications of the exchange of inert gas at the lungs and tissue Pharmacol Rev 3 1 1951

Kitani K Solubility coefficients of ⁸⁵krypton and ¹³³xenon in water saline lipids and blood Scand J clin Lab Invest 29:167 1972

Kitani K, Adgey J, Kirkle E & Møstруп I : Determination of blood flow and lipid content of liver by the simultaneous use of ⁸⁵Kr and ¹³³Ie in patients and isolated pig livers with portal

perfusion J med Med 11:335 1970

Kitami K. & Winkler K : In vitro determination of solubility of 133 xenon and 85 krypton in human liver tissue with varying triglyceride content Scand J clin Lab Invest 29 173 1972

Klatskin G & Rappaport E M. Late residuals in presumably cured acute infectious hepatitis. Ann intern Med 26 13 1947

Klenk H -D. Die Lipidszusammensetzung von normalen und pathologischen Lebern des Menschen pp 42-51. Die Köln 1964.

Kremer G J, Kneeling F K, Lange H J & Victor W. Bestimmung des Fettgehalts in der Leber. Dtsch med Wochr 94 163 1969

Krikler D M & Zilberg B. Activity and hepatitis. Lancet 2:1046 1966

Krook H. Circulatory studies in liver cirrhosis. Acta med scand Suppl 318 1-160 1956

Lang H, Bruck H & Böniach H. Immunisation and excretory function of the liver. Int Arch Allergy 28 336 1965

Lassen W A. Personal communication 1965

Lassen W A & Munck O. The cerebral blood flow in man determined by the use of radioactive krypton. Acta physiol scand 53 30 1955

Laurell S & Lundquist A. Lipid composition of human liver biopsy specimens. Acta med scand 189 65 1971

Levy C M & Gliedman M L. Practical and research value of hepatic-vein catheterisation. New Engl J Med 258 696-700 and 738-742 1958

Lucké B. The pathology of fatal pyrexia hepatitis. Amer J Path 20 471 1944

Mowley J W & Galambos J T. Viral hepatitis. In Schiff L (ed) Diseases of the liver pp 410-497. J H Lippincott Company Philadelphia 1969

- Myhre J R : Assessment of portal venous pressure by hepatic vein catheterization. *Acta med scand* 1,0:281 1954
- Nefzger M B & Chalmers T C : The treatment of acute infectious hepatitis. Ten-year follow-up study of the effects of diet and rest. *Amer J Med* 35 299 1963
- Nelson R S Sprinz H Colbert Jr J W Cantrell F P Havens Jr W P & Knowlton M : Effect of physical activity on recovery from hepatitis. A follow-up study two to three years after onset of disease. *Amer J Med* 16:780 1954
- Neumayr A : Hepatoportal Kreislaufuntersuchungen beim Menschen. *Wien Z inn Med* 37 1 1956
- Nielsen H C : Spectrophotometric determination of indocyanine green in plasma especially with a view to an improved correction for blank density. *Scand J clin Lab Invest* 15:613 1963
- Paton A Reynolds T B & Sherlock S : Assessment of portal venous hypertension by catheterisation of hepatic vein. *Lancet* 1:918 1953
- Preisig R Rankin J G Sweeting J & Bradley S E : Hepatic hemodynamics during viral hepatitis in man. *Circulation* 34 188 1966
- Rappaport A M : Anatomical considerations in Schiff L (ed) *Diseases of the liver* p 1 J B Lippincott Company Philadelphia 1969
- Rappaport A M Knoblauch M Black R G & Chira S : Hepatic micro-circulatory changes leading to portal hypertension. *Ann N Y Acad Sci* 170:48 1970
- Reichman S & Davis Jr W B : The splenic approach to the portal circulation. Intra-splenic and intra-hepatic tissue pressure measurements in acute and convalescent hepatitis. *Gastroenterology* 33 609 1957
- Reichman S Gorlin H Storesli J P & Davis Jr W D : A new method of rapid measurement of hepatic blood flow and portal circulation time employing radioactive indicator dilution technique. *Clin Res Pro* 5 213 1957

- Repsher L.R & Freebern R.K : Effects of early and vigorous exercise on recovery from infectious hepatitis. *New Engl J. Med* 281:1393 1969
- Roholm K & Iversen P : Changes in the liver in acute epidemic hepatitis (catarrhal jaundice) based on 38 aspiration biopsies. *Acta pathol microbiol scand* 16:427 1959
- Rowell L.B Blackmon J.R & Bruce R.A : Indocyanine green clearance and estimated hepatic blood flow during mild to maximal exercise in upright man. *J Clin Invest* 43 1677 1964
- Ruebner B.H & Sussner E.J : Hepatocytes and sinusoidal lining cell in viral hepatitis. *Arch Path* 86 1 1968
- Schaffner F : Intralobular changes in hepatocytes and the electron microscopic mesenchymal response in acute viral hepatitis. *Medicine (Baltimore)* 45:547 1966
- Schmitt W : Die Hämodynamik und der Leberblutdurchfluss bei akuter und chronischer Hepatitis sowie bei Fettleber- und Gallenwegkrankungen. *Z klin Med* 158 85 1964
- Sherlock S : Diseases of the liver and biliary system pp 1-16 and pp 317-349. Blackwell Scientific Publications Oxford, 1968
- Sjstrand T : Functional capacity and exercise tolerance in patients with impaired cardi-vascular function. *Clinical Cardipulmonary Physiology* pp 201-219 Grune & Stratton Inc New York 1960
- Snedecor G.W : Statistical methods 5th ed Iowa State College Press Ames Iowa 1956
- Storti E Lusvardi E Lenzi M Olibertini G Terricelli A Zanbarde E Prati P.L Fontana G Rivi A & Montanari C : La cathétérisme portal Porto-manométrie et porto-hépatographie trans-ombilicale. Remarques physiopathologiques et cliniques. *Presse méd* 74 207 1966
- Strandell T & Lundbergh P : Measurements of ¹³³Xe activity in blood sampled in plastic and glass syringes. *Scand.J clin Lab Invest* 3. 89 1973

Swift Jr W E Gardner H T Moore D J Streitfeld F H & Havens Jr W P Clinical course of viral hepatitis and the effect of exercise during convalescence Amer J Med 8:614 1950

Teodori U & Gentilini P : Aspects ultrastructuraux de l'hépatite anictérique et considérations sur la pathogénèse de l'ictère dans les hépatites Schweiz med Wochr 97:1106 1967

Teodori U Gentilini P & Surrenti C : Electron microscope observations of some forms of viral hepatitis Contribution to the understanding of the morphological evolution and pathogenetic mechanism of jaundice Gastroenterologia (Basel) 108:105 1967

Tønnesen K H & Vejlesen P : Washout of ¹³³xenon after intramuscular injection and direct measurement of blood flow in skeletal muscle Scand J clin Lab Invest 25 71 1970

Viallet A Joly J -O Marleau B & Lavoie P : Comparison of free portal venous pressure and wedged hepatic venous pressure in patients with cirrhosis of the liver Gastroenterology 59:372 1970

Wade O L Combes B Childs A W Wheeler H O Courmand A & Bradley H E The effect of exercise on the splanchnic blood flow and splanchnic blood volume in normal man Clin Sci 15:457 1956

Wahren J Felig P Ahlborg G & Jorfeldt L Glucose metabolism during leg exercise in man. J clin Invest 50:2715 1971

Wick G E Emmett R Craighead C C Hoeffler G Browne D C & Rosen I Simultaneous pressure measurements in the hepatic venule and portal venous system in man Amer J med Sci 228:643 1954

Weppler W & Wildhirt E : Klinische Histopathologie der Leber p 11 G Thieme Verlag Stuttgart 1968

Winkler K Larsen J A Munkner T & Tygstrup N Determination of the hepatic blood flow in man by simultaneous use of five test substances measured in two parts of the liver Scand J clin Lab Invest 17:423 1965

Wild H Hth Org techn Rep Ser No 512 pp 1-51 1973

APPENDIX

Tabl IV Estimated hepatic blood flow (l/min) at rest (RI) t 10 and 15 minute and t the end of two generally 20-minute periods of supine leg exercise (WI and WII) and at 5 10 20 and 30 minutes after the exercise (RII) in 10 patients and 13 control. Data at rest are given for 4 controls in whose values during exercise had to be discarded \bar{X} = group mean value SD = standard deviation n = no of individuals xxx = $P < 0.001$ xx = $P < 0.01$ and x = $P < 0.05$ where P is the probability that the difference between patients and controls was caused by random factors. Figure within parentheses are not included in mean values.

	RI				WI			WII			RII				
		10	15	20	10	15	20	10	15	Final	5	10	20	30	
Patient no	1	1.92	1.70	1.77	1.79	1.40	1.33	1.40	1.66	1.89	2.08	2.08	2.08	2.37	
	2	2.29	2.29	1.97	2.46	1.56	1.45	1.37 ^{a)}	1.65	1.94	2.04	2.04	2.04	2.94	
	3	2.65	2.33	2.52	2.48	2.6	2.51	2.43	2.39	2.74	3.06	2.94	2.94	2.94	
	4	2.71	2.19	2.12	2.09	1.79	1.60	1.37	1.73	1.86	2.08	2.08	2.08	2.47	
	5	2.18	1.77	1.74	1.84	1.42	1.50	1.36 ^{a)}	1.74	1.64	2.03	2.03	2.03	2.31	
	6	2.44	2.24	2.23	1.88	1.26	1.17	1.45 ^{a)}	1.85	1.94	-	-	-	1.83	
	7	2.36	2.47	2.53	2.39	1.90	1.85	1.58	1.83	2.10	2.06	2.06	2.06	2.24	
	8	2.05	1.72	1.63	1.57	1.29	1.10	0.95	1.38	1.60	1.69	1.69	1.69	1.64	
	9	1.83	1.42	1.39	1.29	0.93	0.82	0.78 ^{a)}	1.38	1.43	1.57	1.57	1.57	1.48	
	10	1.69	-	0.98	0.89	0.65	-	0.54 ^{a)}	0.89	1.07	1.22	1.22	1.22	1.39	
	\bar{X}	2.21	2.01	1.89	1.87	1.48	1.48	1.30	1.65	1.82	1.98	1.98	1.98	2.07	
	SD	0.34	0.37	0.49	0.52	0.53	0.49	0.51	0.39	0.44	0.50	0.50	0.50	0.52	
	n	10	9	10	10	10	9	10	10	10	10	10	9	9	
Control no	1	1.43	1.23	1.25	1.20	0.89	0.80	0.63 ^{a)}	1.40	1.54	1.63	1.63	1.63	1.63	
	2	1.49	1.11	1.10	0.89	0.45	0.31	0.28 ^{a)}	0.69	0.69	0.95	0.95	0.95	1.10	
	3	1.46	1.09	1.09	1.08	(0.40)	-	0.50 ^{a)}	0.82	0.97	1.21	1.21	1.21	1.43	
	4	1.31	1.22	1.38	1.32	1.04	1.03	0.80	1.30	1.47	1.39	1.39	1.39	1.40	
	5	1.08	0.78	0.79	0.84	0.41	0.33	0.26 ^{a)}	0.80	0.81	0.92	0.92	0.92	1.28	
	6	1.10	1.30	1.02	0.80	(0.60)	-	0.60 ^{a)}	0.66	0.98	1.09	1.09	1.09	1.28	
	7	1.82	1.30	1.24	1.12	0.76	0.71	0.67 ^{a)}	1.05	1.36	1.52	1.52	1.52	1.64	
	8	1.43	1.35	1.22	1.25	0.85	0.72	0.63 ^{a)}	1.23	1.24	1.20	1.20	1.20	1.38	
	9	1.51	1.30	1.10	1.02	0.68	0.61	0.53 ^{a)}	1.33	1.40	1.25	1.25	1.25	1.56	
	10	1.63	1.35	1.37	1.48	1.18	1.01	0.97	1.58	1.68	1.86	1.86	1.86	1.89	
	11	1.33	1.18	1.21	1.28	0.78	0.67	0.52	1.22	1.30	1.21	1.21	1.21	1.46	
	12	1.66	1.20	1.35	1.27	(0.30)	0.30	0.30	0.96	1.08	1.40	1.40	1.40	1.73	
	13	1.46	1.24	1.27	1.41	(0.62)	-	-	1.03	1.19	1.28	1.28	1.28	1.21	
	14	(1.60)	-	-	-	-	-	-	-	-	-	-	-	-	
	15	(1.38)	-	-	-	-	-	-	-	-	-	-	-	-	
	16	(1.68)	-	-	-	-	-	-	-	-	-	-	-	-	
	17	(1.09)	-	-	-	-	-	-	-	-	-	-	-	-	
	\bar{X}	1.44	1.20	1.18	1.15	0.78	0.69	0.56	1.08	1.21	1.30	1.30	1.30	1.48	
	SD	0.21	0.15	0.16	0.22	0.25	0.24	0.21	0.29	0.29	0.26	0.26	0.26	0.23	
	n	13	13	13	13	9	9	12	13	13	13	13	12	12	
Diff. P	0.77	0.81	0.71	0.72	0.70	0.79	0.74	0.57	0.61	0.68	0.59	0.59	0.59	0.59	
	xxx	xxx	xxx	xxx	xx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xx	

a) Exercise terminated after 10-15 minutes because of fatigue

Table V Oxygen saturation in hepatic venous blood (%) at rest (RI) at 10 and 15 minutes and at the end of two generally 20-minute periods of supine leg exercise (WI and WII) and at 5, 10, 20 and 30 minutes after the exercise (RII) in the patient and the controls. Data at rest are given for 4 controls in whose value during exercise had to be discarded. Figure within parentheses are not included in mean value. Other symbols in Table IV.

		RI			WI			WII			RII			
					10	15	20	10	15	Final	5	10	20	30
Patient no	1	78.1	75.9	77.1	75.9	71.4	72.2	67.3	76.5	75.9	76.7			
	2	79.5	76.8	77.9	74.4	69.0	66.4	66.7	73.2	71.6	71.9	73.4		
	3	77.0	76.1	75.8	75.0	75.6	75.7	6.4	76.5	76.1	73.5	73.8		
	4	86.3	84.8	84.2	84.0	80.4	77.1	75.0	80.8	80.9	82.0	83.4		
	5	80.1	74.9	72.3	73.1	55.4	54.2	49.9 ^a	75	75.4	77.6	79.3		
	6	77.9	74.3	76.0	74.6	60.6	56.8	53.9 ^a	71.2	69.7		69.0		
	7	80.4	81.2	81.2	80.8	74.8	73.9	69.1	74.9	75.8	73.2	76.8		
	8	78.4	68.7	69.8	68.6	58.0	54.3	52.6	65.5	64.5	67.0	68.3		
	9	78.6	71.0	72.6	68.1	40.5	49.2	44.7 ^a	64.4	64.5	68.1	69.7		
	10	75.5	-	73.2	68.6	49.4	-	41.5 ^a	64.2	68.5	71.3	73.2		
	\bar{X}	79.2	76.0	76.0	74.3	64.5	64.4	59.7	72.2	72.3	73.5	74.1		
	SD	2.9	4.8	4.4	5.5	11.1	10.8	12.7	5.8	5.4	4.7	5.0		
	n	10	9	10	10	10	9	10	10	10	9	9		
Control no	1	73.0	-	70.4	67.3	-	43.3	35.9 ^a	65.7	68.0	70.8	70.0		
	2	75.1	-	66.5	67.2	-	8.2	7.6 ^a	35.1	48.4	48.0	59.5		
	3	(77.9)	(68.6)	(64.1)	(62.4)	-	-	-	-	-	-	-		
	4	69.4	69.3	70.6	65.0	60.0	40.9	45.4	70.3	73.3	70.9	74.6		
	5	69.8	59.0	58.7	58.4	30.5	19.9	11.9	48.3	52.3	61.8	70.3		
	6	75.4	80.5	79.9	79.1	(48.6)	-	48.6	74.6	71.7	75.2	79.9		
	7	84.9	82.6	78.8	78.0	58.0	53.3	48.6	70.6	73.6	74.3	75.5		
	8	82.1	78.8	77.1	78.5	62.3	55.7	44.0	73.2	72.8	76.9	77.5		
	9	82.6	73.1	73.4	74.6	51.3	49.6	44.1	72.9	74.1	77.0	81.0		
	10	78.7	74.0	74.4	73.9	71.3	64.1	67.5	71.3	73.2	76.0	79.9		
	11	72.2	66.7	65.7	64.4	46.3	37.0	23.2	59.8	59.0	62.6	65.8		
	12	78.2	72.6	69.5	68.4	(18.0)	-	18.0 ^a	50.5	74.9	80.2	81.8		
	13	74.8	71.5	69.9	68.5	(44.1)	-	-	63.7	67.1	70.1	70.2		
	14	(67.9)												
	15	(72.7)												
	16	(82.1)												
	17	(70.8)												
	\bar{X}	76.4	73.0	71.2	70.3	54.2	42.6	35.9	63.0	67.5	70.3	73.8		
	SD	5.0	7.0	6.0	6.5	13.2	18.2	18.5	12.4	9.3	9.0	6.9		
	n	12	10	12	12	7	9	11	12	12	12	12		
Difference		2.8	3.0	4.8	4.0	10.3	21.8	23.8	9.2	4.8	3.2	0.5		
P		>0.10	0.30	x	0.10	>0.10	xx	xx	x	>0.10	>0.30	0.90		

^a Exercise terminated after 10-15 min ts because of fatigue

Table VI Arterio-hepatic venous oxygen difference (ml/l) at rest (RI) t 10 and 15 minutes and at the end of two generally 20-minute periods of supine leg exercise (WI and WII) and at 5 10 20 and 30 minutes after the exercise (RII) in the patient and the controls. Data at rest are given for 4 control in whom values during exercise had to be discarded. Figure within parentheses are not included in mean value. Other symbols as in Table IV.

	RI	WI		WII		RII					
		10	15	20	10	15	Final	10	20	30	
Pati nt no 1	31.6	37.2	35.6	37.8	47.0	44.7	51.6 ^a	31.9	32.1	29.2	-
2	35.8	42.5	40.9	45.8	57.8	62.6	60.9 ^a	48.4	51.5	48.7	44.0
3	39.3	43.8	44.2	43.9	44.9	44.4	43.0	43.1	42.2	46.2	44.2
4	27.6	31.4	33.6	33.1	41.3	48.9	37.7	39.9	38.0	33.9	31.2
5	37.5	49.6	55.7	49.0	88.3	93.6	102.8 ^a	48.0	45.2	39.7	35.8
6	44.9	54.0	51.0	51.1	82.8	88.6	94.9 ^a	56.4	56.5		54.1
7	31.9	32.0	31.6	31.9	42.1	43.7	50.7	40.7	38.2	41.3	36.0
8	37.8	56.3	53.5	55.5	75.5	82.8	83.8	58.5	60.0	54.6	51.9
9	36.2	50.4	47.2	55.2	89.0	91.8	101.2 ^a	63.1	60.6	52.9	49.8
10	38.2	-	45.2	53.2	93.2	-	106.8 ^a	60.2	51.1	44.7	39.3
\bar{Y}	36.1	44.1	43.9	45.7	66.0	66.8	74.9	49.0	47.5	43.5	42.9
SD	4.8	9.2	8.4	8.8	21.8	22.2	25.3	10.3	9.9	8.4	8.0
n	10	9	10	10	10	9	10	10	10	9	9
Control no 1	48.7	-	57.2	60.5	-	115.2	129.3 ^a	62.4	59.0	48.9	52.1
2	46.3	-	62.1	60.3	-	194.3	191.3 ^a	131.1	91.2	90.1	63.2
3	(44.0)	(64.3)	(64.8)	(70.6)	-	-	-	-	-	-	-
4	56.2	57.1	55.6	67.1	78.6	97.4	109.0	57.6	50	52.5	46.9
5	51.4	80.3	80.7	82.2	142.7	167.7	181.3	102.0	92.1	71.0	54.9
6	50.1	43.0	43.7	46.0	(123.6)	-	123.6 ^a	59.6	61.0	50.2	42.2
7	32.0	38.2	46.2	46.7	86.0	96.7	105.2 ^a	59.2	52.8	51.0	49.1
8	34.0	41.0	44.4	40.3	73.4	87.2	109.4 ^a	48.9	50.1	40.9	38.5
9	33.8	0.8	54.5	52.2	104.1	104.8	117.2 ^a	54.6	51.3	44.2	36.2
10	45.7	48.5	57.8	58.3	68.2	81.3	74.9	63.6	56.8	54.9	45.1
11	37.9	52.9	54.6	57.2	94.0	114.1	138.9 ^a	65.4	65.6	57.0	50.9
12	39.3	51.4	56.3	59.0	(159.8)	-	159.8 ^a	90.8	45.1	34.0	31.2
13	41.1	47.8	50.7	53.3	(99.2)	-	-	62.1	54.7	48.0	47.0
14	(42.2)										
15	(56.7)										
16	(30.2)										
17	(63.0)										
\bar{Y}	43.0	52.1	55.3	56.9	92.4	117.6	130.9	71.4	60.9	53.6	46.4
SD	7.8	11.9	9.8	10.9	25.3	38.2	34.7	24.1	15.4	14.6	8.7
	12	10	12	12	7	9	11	12	12	12	12
Difference P	-6.9 x	-8.0 >0.10	11.4 xx	-11.2 x	-26.4 x	0.8 xx	-56.0 xxxx	-22.4 x	-13.4 x	10.1 >0.05	3.5 >0.30

^a) Exercise terminated after 10-18 minutes because of fatigue.

Table VII Splanchnic oxygen uptake and hepatic extraction of ICG at rest (RI) at the end of two generally 20-minute periods of supine leg exercise (WI and WII) and at 30 minutes after the exercise (RII) in the patients and the controls. Data at rest are given for 4 controls in whom values during exercise had to be discarded. Figures within parentheses are not included in mean values. Other symbols as in Table IV

		Splanchnic oxygen uptake				Hepatic extraction of ICG			
		ml/min				%			
		RI	WI	WII	RII	RI	WI	WII	RII
Patient no	1	60.9	67.6	61.6	-	59.2	54.1	53.1	-
	2	82.9	112.6	83.3 ^{a)}	104.1	57.1	54.1	60.1 ^{a)}	50.0
	3	104.2	108.7	106.3	130.1	37.7	34.0	30.7	26.3
	4	74.6	69.1	73.4	77.1	74.7	73.2	74.3	69.4
	5	81.7	90.1	110.2 ^{a)}	82.4	64.1	60.2	56.8 ^{a)}	49.0
	6	109.4	95.3	137.0	98.8	26.8	23.8	25.0 ^{a)}	22.3
	7	75.2	76.4	80.0	80.4	58.8	47.8	47.4	51.9
	8	77.3	87.0	79.7	85.3	58.4	59.8	66.0	56.4
	9	66.6	71.1	78.6	73.7	57.9	60.8	69.8	54.2
	10	64.5	47.3	57.6	54.7	47.4	56.3	62.6	44.5
		\bar{X}	79.7	82.6	89.8	54.2	52.4	54.6	47.1
		SD	16.0	20.2	28.9	13.6	14.2	16.2	14.7
		n	10	10	10	10	10	10	9
Control no	1	69.8	72.5	80.9	85.0	72.8	61.7	58.5	52.1
	2	68.8	53.5	54.0 ^{a)}	69.8	65.6	68.3	68.6	60.9
	3	(64.4)	(75.9)	-	-	64.6	66.2	76.4	55.4
	4	73.7	88.5	87.5	65.6	80.9	58.7	54.9	56.3
	5	55.4	68.8	47.4	-	72.8	72.3	73.0	-
	6	54.8	36.8	73.9 ^{a)}	54.1	87.0	79.8	82.9 ^{a)}	71.1
	7	58.3	52.4	70.9 ^{a)}	80.7	77.1	80.8	82.0 ^{a)}	69.6
	8	48.8	50.1	68.5	53.0	70.9	60.3	70.8	59.0
	9	51.2	53.2	62.4	56.3	69.7	69.0	73.4 ^{a)}	52.9
	10	74.4	86.3	72.3	85.2	81.0	75.9	77.4	66.5
	11	50.5	73.2	72.4	74.5	73.5	67.2	75.2	58.2
	12	65.2	74.8	47.9 ^{a)}	53.9	73.9	74.9	81.5	54.1
	13	59.8	75.0	-	56.9	88.9	81.0	-	77.8
	14	(67.7)				(77.9)			
	15	(78.1)				(73.1)			
	16	(50.6)				(77.1)			
	17	(68.7)				(63.2)			
		\bar{X}	60.9	65.4	67.1	75.3	70.5	72.9	61.2
		SD	9.2	15.9	12.9	7.5	7.7	8.8	8.2
		n	12	12	11	13	13	12	12
Difference		18.8	17.2	22.7	20.6	21.1	-18.1	18.3	-14.1
P		xxx	x	x	x	xxx	xxx	xx	x

^{a)} Exercise terminated after 10-18 minutes because of fatigue

Table I. Heart rate (beats/min) at rest (RI) at 10 and 15 minute and at the end of two generally 20-minute periods of supine leg exercise (WI and WII) and at 5 10 20 and 30 minutes after exercise (RII) in the patients and the controls. Data at rest are given for 4 controls in whom blood flow values during exercise had to be discarded. Figure within parentheses are not included in mean values. Other symbols as in Table IV

		RI	WI		WII		RII					
			10	15	20	10	15	Final	5	10	20	30
Patient no	1	68	110	110	112	140	140	140 ^{a)}	110	108	75	75
	2	73	123	123	125	145	155	160 ^{a)}	120	105	100	95
	3	71	90	96	93	110	115	120	100	95	90	90
	4	93	120	120	120	160	160	160	120	125	120	120
	5	77	130	126	135	158	180	180 ^{a)}	118	110	113	95
	6	47	110	118	130	165	170	180 ^{a)}	110	100	-	80
	7	63	118	118	113	170	170	174	110	102	96	92
	8	87	129	128	140	173	177	178	122	116	110	106
	9	82	122	122	123	167	168	170	119	118	108	94
	10	91	164	170	168	190	-	194 ^{a)}	130	125	122	118
	\bar{X}	75	122	123	124	158	159	166	116	110	104	97
	SD	14	19	19	20	22	21	22	8	10	15	15
	n	10	10	10	10	10	9	10	10	10	9	10
Control no	1	53	112	112	116	172	175	177 ^{a)}	96	90	73	67
	2	68	108	116	116	178	184	188 ^{a)}	110	102	97	93
	3	63	108	108	114	(185)	185	185 ^{a)}	113	111	97	90
	4	42	102	100	100	147	156	168	90	84	80	65
	5	60	117	118	118	169	176	181	114	108	100	87
	6	56	117	118	118	(193)	-	193 ^{a)}	116	104	89	82
	7	70	123	130	135	170	170	175 ^{a)}	103	102	90	90
	8	60	120	120	125	160	160	165 ^{a)}	110	94	85	83
	9	74	124	124	136	172	175	180 ^{a)}	120	98	88	86
	10	61	84	86	90	132	138	140	76	76	76	70
	11	59	108	108	111	161	165	170 ^{a)}	100	91	86	81
	12	61	121	121	126	(192)	-	192 ^{a)}	110	110	108	103
	13	61	-	100	104	146	(161)	161 ^{a)}	94	88	82	78
	14	(65)										
	15	(56)										
	16	(74)										
	17	(63)										
	\bar{X}	61	112	112	116	161	167	173	104	97	89	83
	SD	8	11	12	13	15	14	15	13	11	10	11
	n	13	12	13	13	10	9	13	13	13	13	13
Difference	14	10	11	10	-3	-8	-9	12	13	15	14	14
	xx	>0.10	>0.10	>0.10	>0.70	>0.30	>0.20	x	xx	xx	x	x

a) Exercise terminated after 10-18 minutes because of fatigue

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Neues über die Wirkungsweise der menschlichen Kohlenmonoxidvergiftung und über die Sauerstoff-Bindungsfähigkeit menschlicher Erythrozyten

Neues klinisches Frühsymptom der Co-Vergiftung

Von K. Lenggrehager

**Neues über die Wirkungsweise
der menschlichen Kohlenmonoxidvergiftung
und über die Sauerstoff Bindungsfähigkeit
menschlicher Erythrozyten
Neues klinisches Frühsymptom der CO Vergiftung**

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I SUMMARY

A number of clinicians attribute a specific effect to carbon-monoxide intoxication in man. Meldrum and Roughton for instance have demonstrated inhibition of carboanhydrase by CO in test tube experiments.

Should this view prove valid, one would expect that recumbent test persons with moderate CO-saturation would develop a considerably diminished tendency to hyperventilation tetany—owing to the inhibition of carboanhydrase. However in a group of 12 normal persons subjected to 25-30% CO-blood-saturation, a series of 6-10 deep breaths in very rapid succession was regularly followed by brief unconsciousness.

This phenomenon cannot be fully explained either by Haldane's CO-O₂-blood saturation curves or by the displacement of the BOHR curves to the left due to the loss of CO₂. Attention is called to the misleading presentation of the CO-O₂-binding curves of blood in Haldane's work. Even in the presence of varying degrees of CO-presaturation all the curves derived from complementary oxygen saturation are plotted against the zero mark of the same curve and not—as might be expected—against values corresponding to for instance the 5, 50 or 75% presaturation levels chosen in the investigation.

In CO-intoxication of man carbon monoxide appears at the upper end of the saturation curve because at this level the least amount of resistance is offered to displacement of oxygen.

Correspondingly CO breathing at rest at high altitudes does not cause symptoms as long as the uppermost, oxygen-free part of the O₂-saturation curve alone is taken up by carbon monoxide. Only when CO-saturation is augmented and oxygen is progressively displaced from the erythrocytes do symptoms of tachycardia and drowsiness occur.

A new discovery is the fact that the onset of hyperventilation-coma occurs earlier at high altitudes than at sea-level in the presence of constant levels of CO-blood saturation. This can be attributed to a specific effect of alkalosis upon the brain.

This effect is enhanced by the strong left shift of the O₂-saturation curve caused by hyper-

ventilation. As measured by modern spectrographic methods this curve also shows a progressive upward trend.

However neither the rectification of the curves nor the modification of O₂-binding due to alkalosis suffices to explain fully the phenomenon of hyperventilation coma.

It was shown that in blood which becomes progressively deficient in CO₂ the binding curve for O₂ moves gradually to the left over the whole scale and not mainly in the medium range. This too has been determined by modern spectrographic CO-oxy-meter measurement.

Thus we are able to understand the mechanism of our experiment in which maximum hyperventilation leads to coma even when blood pressure remains unchanged. For ultimate comprehension of this form of coma and the loss of consciousness brought about by 25% CO-blood-saturation and brief hyperventilation, the following is of importance: cerebral function, mainly as concerns the conscious state, requires a certain elevation of the minimum oxygen tension level in human beings who are not adequately trained or accustomed to alkalosis.

Full understanding of hyperventilation coma in slightly CO-intoxicated persons results from a combination of the following three elements:

1. Reduction of the amount and tension of O₂ in Co-blood.
2. Reduction of tension of O₂ due to alkalosis.
3. Elevation of the minimal O₂-tension threshold necessary for the state of consciousness owing to a detrimental effect of alkalosis.

These new views offer a better explanation for the drastic differences of behaviour in patients with anaemia in the range 50-30% hemoglobin who are still capable of work; this stands in contrast to a 50% CO-blood saturation, which represents the utmost level compatible with the state of consciousness at complete rest and only in a recumbent position.

Finally it has been shown in animal experiments that CO mainly affects the breathing centre rather than the heart. This is why these animals with paralysed respiration recover under O₂ over pressure.

II EINFÜHRUNG

Das internationale Symposium über Kohlenmonoxydvergiftung (Mai 1965) wurde vom Vorsitzenden Symanski mit den Worten eröffnet. „Der Überblick über Schrifttum und Praxis zeigt dass immer wieder unzutreffende Vorstellungen über das Wesen und die Folgeerscheinungen der CO-Vergiftung bekannt werden“

Claude Bernard entdeckte 1857 die grosse Affinität des Kohlenmonoxyds zum Haemoglobin und erklärte den CO-Tod als innere Erstickung was jedoch wegen der zu gross schellenden Verschiedenheit zwischen O_2 -Mangel und CO-Intoxikation stark bezweifelt wurde

Haldane (1895 und 1896) fundierte die Erklärungen Bernards über die Wirkungsweise der akuten CO-Vergiftung, später auch anhand eindrücklicher Selbstversuche Für ihn waren ebenfalls alle Symptome durch den teilweisen Beschlag der Erythrocyte mit CO und der daraus resultierenden zwangsblutigen Hypoxie der Gewebe erklärt.

Während diese Theorie im Verlauf durch die Physiologen wohl allgemeinen Anerkennung fand, stiess sie bei den Klinikern zum grossen Teil auf hartnäckigen Widerstand, indem die Symptome bei CO-Vergiftung zu verschieden und viel zu mannigfaltig schienen gegenüber den reinen Symptomen blossen O_2 -Mangels worauf unter anderem auch Zorn, Strabalin, Moeschlin, Claude Mosinger u. a. hinweisen. Da ich selbst auch der Gruppe von Zweiflern angehörte, unternahm ich zahlreiche Tier und Eigenversuche, bestrebt irgend etwas spezifisch Toxisches für die CO-Vergiftung herauszufinden. Ich möchte ergehennehmen dass mir letzteres nicht gelungen ist, so dass ich jetzt ebenfalls zum überzeugten Anhänger der Haldane'schen Auffassung geworden bin allerdings mit einer neuen wesentlichen Modifikation weshalb mir die Veröffentlichung und die

etwas längere Einleitung dieser Arbeit gerecht fertigt erscheinen.

Ausgangspunkt bildete zunächst folgende Fragestellung: Warum werden Menschen bei protrahierter Atmung von O_2 -armer Luft häufig tetanisch oder praetetanisch während dies bei der CO-Vergiftung kaum vorzukommen scheint oder jedenfalls sehr selten ist (Zu nach Henderson aus dem Handbuch der Speziellen pathologischen Anatomie und Histologie 1958) Wohl aber finden sich bei der CO-Vergiftung hypotische Krämpfe und sehr viele tonisch-krampfartige Rigorzustände

Könnte also die praktisch fehlende Tetanie bei der CO-Vergiftung ev. auf Hemmung der Carbanhydrase durch CO beruhen wie dies Meldrum u. Roughton (1934) glauben?

In einem Eigenversuch wurde ich in einer Unterdruckkammer entsprechend einer Höhe von 9000 m ü. M. ohne Benützung von Sauerstoff tetanisch (Diesen Versuch, der 45 Minuten dauerte, erdanke ich Herrn PD Dr. F. von Tavel welcher in seiner Unterdruckkammer die Fliegererregungsprüfungen leitete) Durch Atmen von CO_2 ging die Tetanie jeweils zurück, am wenige Minuten nach Sistieren der CO_2 -Atmung infolge des vergrösserten Atemvolumens wiederum zu erscheinen. Hier hatte also der O_2 -Mangel in der Atmungs-luft genügt, um über den Weg erstarrten Atmens zu einem konsekutiven CO_2 -Verlust zu führen. Nun aber bewirkt das Anwesenheit des Fermentes Carbanhydrase dass (bei erhöhtem Abbruch von Blut- CO_2) aus dem physiologischerweise im Blute vorkommenden Natriumbicarbonat ($NaHCO_3$) unter Abspaltung von CO_2 das stark alkalisch reagierende Na-Karbonat (Na_2CO_3) entsteht. Letzteres führt aber nach eigenen Untersuchungen über den Weg verminderter Calciumionisierung zur Tetanie (Lenggenhager 1951) So findet man nach kürzerer aber stärkster Hyperventilation schon Verschiebungen des Blut-pH um 0.15-0.25 nach der alkalischen Seite! Durch blosses Abatmen von gelöster CO_2 ohne die Carbanhydrase wäre nach chemischer Vorstellung nicht mit einer merklichen Änderung der Reaktion (pH) zu rechnen. Auch von Schroeter (1904), Fleming (1911) und Barcroft (1927) fan-

Während 16 Jahren (1942-1960) haben wir während der Frühkaspern jeweils 14-tägige höhenphysiologische Studien im Forschungsinstitut auf Jaegerwisch (3400 m) mit je 1.-14 Teilnehmern durchgeführt. Ein kleiner Bruchteil der Ergebnisse findet in dieser Arbeit seinen Niederschlag.

den in grossen Höhen tetanische Erscheinungen (zit bei Loewy S 767)

Umgekehrt sind also bei CO-Vergiftung praktisch keine rein tetanischen Zustände (wohl aber hypoxische Krämpfe) bekannt.

Damit schuen aber klinisch eine gewisse Möglichkeit zu bestehen anhand dieser Carbinhydrase-Hemmung durch CO eine allfällig verzögert auftretende Hyperventilationstetanie im Eigenversuch zu erhalten verglichen mit vorgängigen Hyperventilationstetanie Zeiten vor der CO-Atmung. Da (durch eigene Methode) mein Schüler Stirnmann eine Teilentkalkisierung des Blutes während Hyperventilationstetanie nachweisen liess (unter Konstanz des absoluten Ca-Gehaltes) wollten wir also eine verminderte Calcium-Teilentkalkisierung bei CO-Versuchspersonen im Hyperventilationstest nachweisen.

Ruhe Hyperventilation bei CO-Vergiftung

Nachdem ich während 35 Minuten ein 5%iges CO-Luftgemisch in liegender Lage geatmet hatte betrug meine Blut-CO-Sättigung 30% (Die Blut-CO-Bestimmungen wurden im Physiologisch-Chemischen Laboratorium durch Prof J Abelein Ordifür physiologische Chemie persönlich aus-

geführt) Der Ruhepuls war von 60 auf 76 gegangen, die Gesichtshaut gerötet. Es bestanden Zeichen von ganz leichtem Schwindel, Euphorie und Dösigkeit.

Jetzt begann ich liegend gegen gewöhnliche Zimmerluft maximal aktiv zu hyperventilieren in der Vorstellung die sonst bei mir so typische Überatemungstetanie nicht mehr (oder stark verspätet) zu erhalten. W7 erstaunt waren wir alle als ich schon nach den ersten 8 tiefen Atemzüge welche innerhalb von 10 Sekunden erfolgten plötzlich und ohne jegliche Vorboten bewusstlos wurde und Krämpfe und Zuckungen bekam. Nach ungefähr 10 Sekunden war ich wiederum bei Bewusstsein.

Ich war mit dem Versuch. Es genügte sogar 6 tiefe Atemzüge um ca 3 Sekunden später wieder zu erwachen. Der vorübergehende Bewusstlosigkeit war um Krämpfen zu erzeugen. Ein Hyperventilationstest mit reinem O₂ führte dagegen innerhalb von 5 Sekunden zu den gleichen Erscheinungen. Bei meiner M.L. bei einem ebenfalls zu diesem Versuche bei mir wurde das gleiche Phänomen wie bei mir beobachtet.

Blut-CO-Gehalt von 28-35% auf. Alle mussten ebenfalls ca 35-40 Minuten lang ein 5%iges CO-Luftgemisch liegend atmen bis die 30%ige Blut-CO-Sättigung erreicht war (Insgesamt 18 Versuche mit 12 Versuchspersonen).

Sofort anschliessende gleichstarke Hyperventilationen derselben CO-Versuchspersonen mit einem 5%igen CO₂-Luftgemisch machten während 3-5 Minuten keinerlei pathologische Symptome (es wurde nicht länger geprüft). Dagegen stellten sich bei absichtlicher weiterer reiner Luft Hyperventilation die beschriebenen, schweren Zustände wiederum ein. Mit zunehmender Wartezeit (in Liegelage) konnten diese Hyperventilations-Ohnmachten zwar noch nach 1 Stunde ausgelöst werden bedurften aber einer etwas längeren Hyperventilation von 15-18 Sek. Dauer. Die Dauer dieser kurzen, mit klonischen Zuckungen einhergehenden Ohnmachten betrug je nach Versuchsperson 10-20 Sek.

Es mag zunächst überraschend erscheinen und hat mich umso mehr überrascht, dass es zur Erreichung von nur 30-35% Blut-CO-Sättigung eines so kurzen Atemungsversuches mit so hoher 5%iger CO-Beimischung bedarf während in den toxiologischen Büchern (z.B. Flury und Zerkel (1931), Teletzky (1955) und andere) diese Konzentration als tödliche Grenze angegeben wird. Nach Teletzky führt Einatmung von 0.1% CO-Luft zu 51.5%iger Blut-CO-Sättigung, ad damit zu Synkope. Es hängt dies sicher damit zusammen, dass wir ausgetrübte, aber sehr Versuchspersonen mit minimalem Ruhe-Atemvolumen waren. Im Gegensatz hierzu befällt die CO-Vergiftung des Alltagslebens werktätige Menschen mit grösserem Atemvolumen. Auch die Selbstversuche durch J. S. Haldane (1893) wurden im Sitzen ausgeführt. Es genügte hierbei schon eine CO-Beimischung von nur 0.4% zur Erzielung einer Blut-CO-Sättigung von 30% wobei (wohl wegen des Sitzens) eine Vergrösserung des Atemvolumens erreicht wurde. Allerdings dauerten die Einatmungsversuche dann ein Vielfaches länger ca halbstündigen Einatmungsversuche.

Als Nebenbeobachtung sei erwähnt, dass unsere Minutenatemvolumina nur während des mittleren Drittels dieser Versuche mit CO-Einatmung leicht vergrössert wurden. Im Durchschnitt blieben die Atemvolumina in der Norm. Es soll später noch näher darauf eingegangen werden.

Damit aber haben wir mit uns ein Hyperventilationstest in liegender Ruhelage vermutlich ein Frühstadium leicht CO-ergiftig erreicht. Es fand die allsehr rasch eine tiefe Ohnmacht nach einem 6 tiefen raschen Einatmen.

wöhnlicher Luft erfolgenden Atemzügen in Liege-
lage (in je 1 Sek. 1 tiefste In- und Expiration!)

Nach Zorn ist sonst leichte CO-Vergiftung nur
über den Weg mühsamer Blutgasanalysen erfass-
bar. Die beschriebene Test füllt damit eine
Lücke in der Früherkennung leichter CO-Ver-
giftung.

Diese Zustände sind nach eigenen Untersu-
chungen genau gleich wie die durch bewusste
Hyperventilation in reinem Stickstoff sich so bald
einstellenden hypoxischen Krämpfe. Sie müssen
ebenfalls als hypoxisch bedingt aufgefasst werden,
da sie bei Hyperventilation gegen reinen O_2 er-
spätes auftraten. Durchschnittlich in 80–120 Se-
kunden gegenüber 8–15 Sekunden bei Lufthyper-
ventilation. Das Erstaunliche dabei aber war die
Tatsache, dass die Ohnmacht also selbst bei reiner
 O_2 -Hyperventilation noch auftrat. Auch dieses
regte zur weiteren Erforschung mächtig an.

Der Unterschied zwischen Haldane im Sitze trotz
einer Blut-CO-Sättigung von rund 30 und mehr Prozent
durch die hierbei leicht ergrünzte Atmung ohne eben
beschriebenen O_2 -Mangelkrämpfe nicht bekam, beruht
sowohl auf dem im Sitze nur leicht gesteigerten Atemvolumen
und der durch die Muskelrelaxation im Sitze vermehrt
gebildeten Muskelmilchsäure. Deswegen konnte ich
15 Minuten nach Beendigung eines 35 minütigen CO-
Atemungsversuchs (von 5% CO in der Atemluft)
zwei oder drei Atemzüge in einem Fahrrad rasge
Zeit herumdrehen, ohne jedoch die Hyperventilations-
anfälle zu bekommen, welche sich bei Hyper-
atmung im Liegen so prompt einstellen.

Durch die Sättigung und die hierdurch bedingte
grosse Atmung wurde ein Großteil des Blut-CO aus-
getrieben, sodass nach dieser Leistung subjektives
Wohlfühl empfundener wurde mit Ausnahme leichter
Kopfschmerzen.

Aus den beschriebenen Hyperventilationscoma-
ständen bei nur 25%iger CO-Blutsättigung ergibt
sich zum 1. die Tatsache, dass durch eine 30%ige
CO-Blutsättigung je nach der C-Dehydratase in
keiner Weise gehindert ist.

Zudem ergaben spätere Untersuchungen meines
Schölers Schlapfer, dass erst durch 50%ige CO-
konzentration in Luft (entsprechend einem Druck
von 1 Atmosphäre) eine erste, kleine Hemmung
der Carbanhydrase feststellbar wurde, gemessen
an der ersaturierenden Alkalität einer während 20'
in Zimmerluft gestandenen 0,1%igen $NaHCO_3$ -
Lösung mit und ohne Carbanhydrasezusatz. Be-
denkt man aber, dass es zur schweren CO-Ver-
giftung des Menschen weniger als 1 mmHg CO-
Druck braucht, so ergibt sich die riesige Über-

dosierung des CO im erwähnten Versuch mit
seinem ungefähr 360 mmHg betragenden CO-
Druck (bei einem Barometerdruck von 720 mm).
Man bedenke hierbei, dass schon ein um nur das
3–4fache gesteigerter O_2 -Druck innerhalb we-
niger Tage für den Menschen und Säugtiere
tödlich ist. So starben z.B. unsere 10 Meer-
schweinchen, die wir auf Jungfranjoch reinen O_2
bei einem Barometerdruck von nur 490 mm atmen
liessen im Verlauf von 6 Tagen, während die Kon-
trolltiere die Jochluft atmeten gesund blieben.
So begreift man auch, dass eine Druckerhöhung
des schädlichen CO um mehr als das 360-fache
noch schädlicher sein dürfte.

Die prompte Wirkungsweise unserer äusserst
kurzen Hyperventilationsversuche bei nur mittleren
Graden von CO-Vergiftung (alle Versuchspersonen
waren gefühlig und schrieben fehlerfrei) weist
aber nun, wie mir scheint, mit besonderer Deut-
lichkeit und vermutlich diesem Tatsachenmaterial
in eine neue Richtung für die Erklärung der CO-
Vergiftung.

Haldane: Erklärung der CO-Wirkung

Nach eigenen Versuchen von Haldane ging es
zunächst darum zu erklären, warum der mensch-
liche Körper mit nur 50% CO-Gehalt seiner
Erythrocyten schwerst geschädigt ist, nahe an der
Grenze des Bewusstseins lebt und bei kleinsten
taumelnden Arbeitsversuchen zu Ohnmacht neigt
(vergl. dazu den Haldane'schen Eigenversuch mit
46%iger Blut-CO-Sättigung). Im Gegensatz hierzu
werden chronische Anaemien von 50% Hb und
weniger erstaunlich gut vertragen.

An mir selbst habe ich die Haldane'schen An-
gaben voll bestätigen können. Indem ich bei 55%
iger Blut-CO-Sättigung mehrmals bei Gehver-
suchen kollabierte und vorübergehend jeweils be-
wusstlos wurde. Dabei hatte ich jedesmal das
einer orthostatischen Ohnmacht vorausgehende
unangenehme Übelkeitsgefühl.

In Liegelage mit passiver Beinhochlage erholte
ich mich jeweils rasch und konnte zwar zitterig,
aber fehlerfrei schreiben wie z.B. in Abb. 1.

Zur Erreichung dieser hohen Blut-CO-Sättigung
musste ich ein 7,5%iges (?) CO-Luftgemisch
während 10 Minuten in liegender Lage einatmen.

Im grossen Gegensatz hierzu weiss man aber,
dass ein 50%iger Hämoglobilverlust bei chroni-
scher Blutarmut sich im Alltagsleben kaum aus-
wirkt (erst bei grösserer Arbeitsleistung).

daher schon Darstellung zuerst nahezu senkrecht ansteigen, um oben fast rechtwinklig und normal weit nach rechts hinüberreichend zu verlaufen.

Es ist dies aber meines Erachtens ein Trugschluss bedingt durch die Darstellung der Haldane'schen Abb 2 trotz der II-CO-Sättigung der Erythrocyten gleichbleibenden Massstäbe für die Darstellung der weiteren prozentualen Aufsättigungskurven durch O₂. Ist jedoch ein Erythrocyt z. B. mit 25% CO vorgefüllt, so darf die weitere Auffüllung mit O₂ nicht mehr bei „Null“ der Abb 2 starten, sondern bei 25% der Ordinate für ein 50%iges CO-Blut bei 50 und für ein 75%iges bei 75 usw.

Alle Werte, die in der Abb 2 z. B. auf der O₂-Bindungskurve der 50%igen CO-Vorsättigung liegen müssen also immer reduziert werden durch den Wert $(94-50)/94$ wobei 94 das in der Haldane-Kurve bei 100 mm O₂-Spannung erreichte Maximum der O₂-Sättigung (entsprechend der Abb 2) darstellt. Dieser Faktor ergibt 0,468.

Diese O₂-Sättigungskurve muss wegen der 50%igen Vorfüllung durch CO also bei 50% der Ordinate beginnen!

Für die 25%ige CO-Kurve ergibt sich ein Reduktionsfaktor von $(93-25)/93$ was einem Wert von 0,731 entspricht. Diese Sättigungskurve der Haldane'schen Abb 2 muss nach Reduktion ihrer Werte bei 25% gestartet werden. Für die 75%ige CO-Kurve endlich ergibt sich ein Reduktionsfaktor von $(93-75)/93$ was dem Wert 0,21 ergibt. Alle Messpunkte der Original-CO-O₂-Kurve des zu 75% oxygenisierten CO-Blutes (Abb 2) sollen also um 0,21 reduziert werden und ihr Anfang bei 75% beginnen.

Jetzt erschien mir ein- und derselben Darstellung sowohl die Spannungsverhältnisse als auch die absoluten Mengen des an die CO-Erythrocyten gebundenen O₂ sofern die Messpunkte dieser Kurven als horizontale Abszissen verlängert gedacht werden (Abb 3).

Würde jedoch eine 50%ige CO-Vorsättigung des Blutes den 30 mm Ladedruck bedingen, welcher für eine 50%ige O₂-Sättigung dieses Normalblutes nötig ist, so könnte die weitere O₂-Sättigung dieses 50%igen CO-Blutes erst erfolgen bei Drucken die höher sind als 30 mmHg. Dann erst wäre die Haldane'sche Vorstellung richtig, dass es sich um CO-Erythrocyten bei Totalaufsättigung mit O₂ um normale O₂-Spannungsverhältnisse handeln würde.

Nun darf man aber den allerersten O₂-Sättigungswerten in stark CO-haltigem Blut, welche nach der Haldane'schen Methode be-

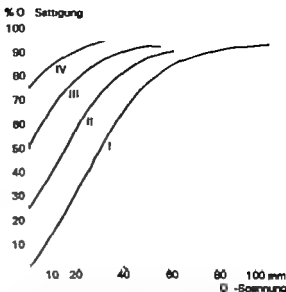


Abb 3 Zeigt die einem einheitlichen Massstab angepassten Sättigungskurven der Abb 2. I=Normal-Kurve der O₂-Sättigung vom Blut II=25% CO-Vorsättigung, dann O₂-Nachsättigung. III=50% CO-Vorsättigung, dann O₂-Nachsättigung. IV=75% CO-Vorsättigung, dann O₂-Nachsättigung.

stimuliert werden kein grosses Gewicht mehr beimessen da diese Methode mit je nur 1-2 ccm Blut arbeiten muss weil durch grössere CO-Blutmengen nach der Gasaustrübung nach Haldane's Angaben eine Teilrückabsorption des ausgetriebenen Gases erfolgt. Infolge der überaus kleinen O₂-Volumina der ausgetriebenen allerletzten O₂-Prozente aus CO-teilgesättigtem Blut sind diese obersten Kurvenabschnitte eher spekulativ. Es wird später darauf eingegangen. Damit erscheint die Ansicht einer normalen O₂-Endspannung in CO-haltigem Blut rein theoretisch.

Nun gibt es aber noch 2 Gründe welche gegen die Ansicht einer normalen O₂-Spannung im teilbeladenen CO-Blut nach Auf sättigung mit O₂ bei normalem Luftdruck sprechen. Erstens haben wir in unseren 12 Versuchen mit 8 Versuchspersonen welche ausgeruht im Liegen ein 5%iges CO-Luftgemisch atmeten schon nach 7 Minuten einen Pulsanstieg von 59 auf 67 bei noch völligem subjektivem Wohlbefinden festgestellt. Dieses Frühwarnzeichen wäre unverständlich bei der Vorstellung einer normalen O₂-Spannung.

Zweitens wurde auch das Rubemuntenatemvolumen nach 7 Minuten und während der nächsten 21 Minuten in diesen Versuchen leicht

Atemvol
Liter/Min

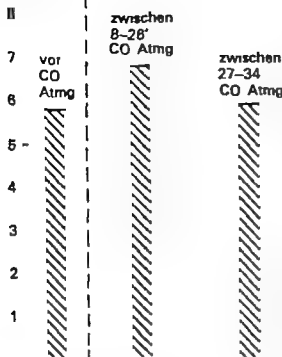


Abb. 4 zeigt, dass am Ende einer 34minütigen 5%igen CO-Atmung (Maskenatmung) das Atemvolumen nicht mehr erhöht ist. Durchschnittswerte von 5 Personen (Durchschnittsalter 29 Jahre).

erhöht (S. Abb. 4). Auch dies bedeutet eine bald auftretende Stressreaktion, die noch kaum subjektiv wahrgenommen wird. Wiederum wäre dies beim Vorliegen normaler O_2 -Spannung nicht erklärbar.

Als weiteren Test für die Richtigkeit dieser Überlegungen erblickte ich auch folgendes: zeichnet man die nach besprochener Weise reduzierte O_2 -Sättigungskurve eines 50%igen CO-Blutes der Abb. 3 auf Seidenpapier so lässt sich diese Kurve in nahezu idealer Weise mit der oberen Hälfte der normalen O_2 -Sättigungskurve (Kurve I der Abb. 1) in Deckung bringen, sofern die reduzierte Kurve bei 50% der normalen O_2 -Sättigung startet. Hierbei muss die Kurve um 30 mm O_2 -Spannung nach links verschoben werden, wobei jeder Punkt auf der Kurve mit der Normalenkurve übereinstimmt. Die Verschiebung von 30 mm Hg auf der

Ähnliche Verschiebung ist auch bei anderen CO-Konzentrationen beobachtbar. Offenbar benutzte aber II

zu 28% mit CO vorgesättigtes Blutprobe anstelle einer 5%igen, was aus der guten Überlagerungsmöglichkeit hervorgeht.

Dagegen ergeben sich aus der modifizierten 75% CO-Kurve nur noch andeutungsweise die obersten 25% einer O_2 -Sättigungskurve. Dies ist wohl bedingt durch die erwähnte Tatsache, dass immer schwieriger werdenden Bestimmungen sehr kleiner O_2 -Beträge im Endteil der Sättigungskurve, wenn man bedenkt, dass Haldane in seinem Apparat nur je 1 (2) ccm Blut pro Analyse benutzen durfte.

Auch folgende Methode rechtfertigt die beschriebene Auffassung einer zu reduzierenden Darstellung der O_2 -Bindungskurve eines teilweise mit CO vorgesättigten Blutes. Alle horizontalen Verbindungslinien zwischen Kurve I, II und III der Abb. 3 weisen (mit Ausnahme der allerersten, weil ungenaueren Abschnitte) nahezu überall den gleichen Betrag auf. Dieser beträgt für das 25%ige CO-Blut ca. 15 mm O_2 -Spannung, für das 50%ige ca. 30 mm.

Damit ist also z.B. die O_2 -Bindungskurve des 50%igen CO-vorgesättigten Blutes ganz einfach um den Wert von 30 mm Spannung nach links verschoben. Sie stimmt sich also überall bei um 30 mm vermindelter O_2 -Spannung auf, ergiebt sich mit der normalen Blutsättigungskurve (vgl. Abb. 5).

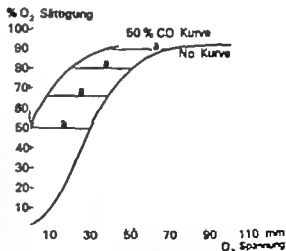


Abb. 5: Obere Kurve: O_2 -Bindungskurve in 50% CO-Blut bei 40 mm CO_2 -Spannung. Untere Kurve: normale O_2 -Bindungskurve bei 40 mm CO_2 -Spannung. Die Querabstände a bleiben sich gleich bis zu 90%iger O_2 -Sättigung. Gleiche Sauerstoffsättigungen geschehen also in diesem CO-Blut praktisch immer schon bei um 30 mm niedrigeren O_2 -Spannungen.

Man könnte diesen Überlegungen den Vorwurf blosser Spekulation machen. Deshalb liess ich diese Frage durch meinen Schüler Spycher in einer Inauguraldissertation anhand des modernsten spektrographischen Instrumentes des „CO-Oxymeters M 182 der J.L. inc. Lexington Mass.“ überprüfen. Das Instrument analysiert automatisch bei 38°C. Eine peristaltische Pumpe saugt anaerob die Blutflussigkeit auf, hämolyisiert die Probe und bringt sie in die Mess-Cuvette. Über den Weg der spektroskopischen Absorption registriert das Gerät simultan die Blutprobe bei 3 sorgfältig ausgewählten Wellenlängen im grünen Spektralbereich. Dies gestattet die prozentuale Feststellung des Oxyhämoglobins CO-Hb und des totalen Hämoglobins in ein und derselben Probe.

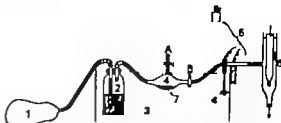


Abb. 6 1 Gasgemisch von 150 l im Gummisack 2 Wasserflasche zur Konstanthaltung der H_2O -Spannung 3 Wasserbad von 38°C 4 Schüttelkolben von 200 cc Inhalt mit jeweils 4 cc Blut; 5 Heizung durch Thermostat mit Rührwerk 6 Wasserstrahlpumpe 7 Blut, A=Hahn zur Blutentnahme; B=Hahn für Gasfüllung des Schüttelkolbens.

Vorgehensweise für die Herstellung der gesättigten $CO-O_2$ -Blutgemische

Alle Versuche wurden im Wasserbad bei 38°C durch meinen Schüler Spycher durchgeführt (s. Abb. 6). Da die Blutgasättigung stark temperaturabhängig ist, muss ein Thermostat mit Rührwerk für Temperaturkonstanz sorgen.

Damit infolge der relativ hohen Temperatur und des vorbeistromenden, trockenen Gasgemisches kein Eindringen des Versuchsbldes eintritt, wird das Gasgemisch durch eine dazwischen geschaltete Wasserflasche (2 der Abb. 6) geleitet.

Der Schüttelkolben ist an eine Wasserstrahlpumpe angeschlossen, die ein dosiertes Durchströmen des Kolbens ermöglicht und durch den Hahn B abgesperrt werden kann. Hahn A ermöglicht das Einfüllen und Entnehmen der Blutproben.

Versuchsvorgehen

1 ca. 20 cc heparinisiertes menschliches Frischblut, unmittelbar vor Versuchsbeginn entnommen, wird in einem Erlenmeyerkolben von 100 cc durch Schütteln ständig zugeführt, reinem N₂ reduziert.

2 Herstellung der Gasgemische

a) für CO -Sättigung des Blutes. In einem evakuierten 200 l fassenden Gummisack werden total 150 l gemischt.

Herrn Prof. Dr. H. P. Gurtner, Leiter der kardiologischen Abt. des Inselspitals, sei auch an dieser Stelle herzlich gedankt für die Erlaubnis, mit diesem Apparat arbeiten zu dürfen.

150 mm Spannung CO , 40 mm Spannung CO_2 werden ergänzt auf 150 l mit reinem, O_2 -freiem Stickstoff.

Mit diesem Gasgemisch werden zunächst ca. 20 cc heparinisiertes Blut bei 38°C entspiert. Abb. 6 zeigt CO gesättigte Kontrollmessungen im CO -Oxymeter ergaben 100% CO -Sättigung.

b) In einem 2. Gummisack werden Gasgemische der gewünschten O_2-CO_2 -Spannung nach folgender Formel hergestellt:

gewünschte Spannung von O_2 oder CO_2	$\frac{z.B. 50 \text{ mmHg}}{z.B. 720 \text{ mmHg}}$	$\frac{x l}{150 l} \rightarrow x$
jeweiliger Barometerstand am Versuchstag		

$$= \frac{150 \cdot 50}{720} = 10.416 l$$

3 Beispiel für ein 50%iges CO -Blutgemisch mit nachfolgenden O_2 -Teilsättigungen bei 40 mm CO

Bei 38°C wird zuerst mittels Wasserstrahlpumpe aus dem mit dem jeweiligen O_2-CO_2 Gasgemisch vorbereiteten Gummisack das gesamte System der Abb. 6 vorgefüllt. Hernach wird Hahn B geschlossen. Der Gummisack wird nun so unter Druck gesetzt, dass beim Öffnen des Einfüllhahnes A der Abb. 6 nur Gas entweichen, aber keine Aussenluft eindringen kann.

Mittels Spritze und feinem Katheter werden 2 cc CO -Blut und sofort 2 cc reduziertes Normalblut eingefüllt. Nun wird 10' lang abwechselungsweise das Blut kräftig in dem Kolben geschüttelt (wobei

Atemvolum
Liter/Min

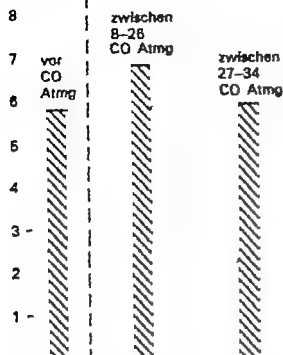


Abb 4 zeigt, dass am Ende einer 34-minütigen 5%igen CO-Atmung (Alaskenatzug) das Atemvolumen nicht mehr erhöht ist. Durchschnittswerte von 5 Personen (Durchschnittsalter 29 Jahre).

erhöht (S. Abb. 4). Auch dies bedeutet eine bald auftretende Stressreaktion, die noch kaum subjektiv wahrgenommen wird. Wiederum wäre dies beim Vorliegen normaler O_2 -Spannung nicht erklärbar.

Als weiteren Test für die Richtigkeit dieser Überlegungen erblicke ich auch folgendes. zeichnet man die nach besprochener Weise reduzierte O_2 -Sättigungskurve eines 50%igen CO-Blutes der Abb. 3 auf Seidenpapier so lässt sich diese Kurve in nahezu idealer Weise mit der oberen Hälfte der normalen O_2 -Sättigungskurve (Kurve I der Abb. 3) zur Deckung bringen, sofern die reduzierte Kurve bei 40% der normalen O_2 -Sättigung beginnt. Hierbei muss die Kurve um 30 mm Hg nach rechts verschoben werden, wobei jeder Punkt der Kurve mit der Normalkurve bei gleicher O_2 -Spannung übereinstimmt.

Ähnliche Verschiebungen sind aber auch bei der 25%igen O_2 -Sättigung beobachtet worden. Offenbar besteht eine

zu 28% mit CO vorgesättigte Blutprobe anstelle einer 25%igen was aus der guten Überlagerungsmöglichkeit hervorgeht.

Dagegen ergeben sich aus der modifizierten 75% CO-Kurve nur noch anderungsweise die obersten 25% einer O_2 -Sättigungskurve. Dies ist wohl bedingt durch die erwähnte Tatsache, dass der immer schwieriger werdenden Bestimmungen sehr kleiner O_2 -Beträge im Endteil der Sättigungskurve wenn man bedenkt, dass Haldane in seinem Apparat nur je 1–2 ccm Blut pro Analyse benutzen durfte.

Auch folgende Methode rechtfertigt die beschriebene Auffassung einer zu reduzierenden Darstellung der O_2 -Bindungskurve eines teilweise mit CO vorgesättigten Blutes. Alle horizontalen Verbindungslinien zwischen Kurve I II und III der Abb. 3 weisen (mit Ausnahme der allerersten, weil ungenaueren Abschnitte) nahezu überall den gleichen Betrag auf. Dieser beträgt für das 25%ige CO-Blut ca. 15 mm O_2 -Spannung, für das 50%ige ca. 30 mm.

Damit ist also z. B. die O_2 -Bindungskurve des 50%igen CO-vorgesättigten Blutes ganz einfach um den Wert von 30 mm Spannung nach links verschoben. Sie fällt also überall bei um 30 mm verminderter O_2 -Spannung auf verglichen mit der normalen Blutsättigungskurve (vergl. Abb. 5).

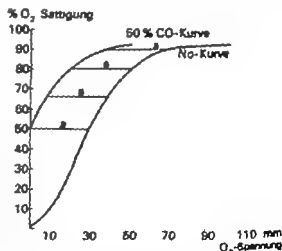


Abb. 5 Obere Kurve: O_2 -Bindungskurve in 50% CO-Blut bei 40 mm CO_2 -Spannung. Untere Kurve: normale O_2 -Bindungskurve bei 40 mm CO_2 -Spannung. Die Querabstände a bleiben auch gleich bis zu 90%iger O_2 -Sättigung. Gleiche Sauerstoffsättigungen geschehen also in diesem CO-Blut praktisch immer schon bei um 30 mm niedrigeren O_2 -Spannungen.

O Sättigung

in %

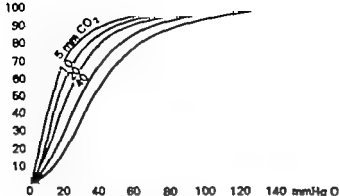


Abb 7 Prozentuale O_2 -Sättigungskurven von Blut bei $38^\circ C$ und verschiedenen CO_2 -Spannungen (nach Bohr, Hasselbalch und Krogh)

kurve nach links verschoben, im Sinne der leichteren O_2 -Bindung aus Hämoglobin (s. Abb 7). Dadurch werden dem Gewebe rund 10 mm O_2 -Spannung entzogen, und die neue O_2 -Sättigungskurve identifiziert sich nahezu mit der 50% CO_2 - O_2 -Kurve. Der resultierende Zustand entspricht nun also einem zu 50% mit CO vorgesättigten und dann durch O nachgefüllten Blut entsprechend der Kurve IV der Abb 2 im Zustande ohne Hyperventilation wobei jedoch noch keine Bewusstlosigkeit im Liegen auftritt. Dadurch wird aber keine Erklärung gegeben für das prompte Koma bei kurzer Hyperventilation im Zustand eines nur 25%igen CO -Blutes.

Ein Versuch zur Deutung des Hyperventilationskomas anhand der reduzierten CO - O_2 -Bindungskurven entsprechend der Abb 3 mislingt ebenfalls, indem durch hyperventilatorische Alkalose die nach links erschoebene Kurve II der Abb 3 nicht einmal die Kurve III erreicht. Diese aber entspricht dem Zustande eines zu 50% CO -beladenen Blutes, was noch kein Koma im Liegen bedingt. Diese Linksverschiebung der O_2 -Bindungskurven durch den CO_2 -Verlust (nach Bohr) sind in Abb 7 ersichtlich.

Es müssen also zur Erklärung andere Überlegenheiten geltend gemacht werden und nicht CO -vorbehandeltes Blut (in den Haldane sehen Versuchen) für die restliche O_2 -A-Sättigung benutzt, sondern umgekehrt ein mit normalem O_2 -Druck an Luft vorgesättigtes Blut das einer progressiven CO -Konzentration ausgesetzt wird.

So werden die Verhältnisse bei der menschlichen CO -Vergiftung imitiert, indem bei zunächst normaler Blut- O_2 -Spannung auch kleinste Drucke an CO einschleichen.

Hierbei aber ist meiner Meinung nach zu erwarten, dass diese kleinen CO -Spannungen zuerst progressiv den noch durch O_2 frei gebliebenen Teil der Sättigungskurve auffüllen also die obersten noch leeren Teile der Hb-Bindungskurve belegen wo noch kein O_2 eingetreten ist und infolgedessen dem CO -Eintritt noch kein Konkurrenzkampf mit dem O_2 bevorsteht. Es erinnert diese Situation an den in der ganzen Naturwissenschaft gültigen Satz des „geringsten Zwanges“ (nach Le Chatelier, Eggert, Carnot, Helmholtz): Mit zunehmender CO -Spannung wird also der O_2

„von oben her“ und zwar progressiv schwerer ausgetrieben weil dieser nach unten immer stärker am Hämoglobin haftet. Dies ist in der Abb 8 für ein 25% CO -nachgesättigtes Lungenblut dargestellt. Haldane hat selbst solche Bestimmungen der CO -Bindungsfähigkeit von vorgängig durch O_2 „on round 1“ einer Atmosphäre (also bei normalem O_2 -Druck vorgesättigtem Blut) vorgenommen. Er hat gefunden, dass jetzt die CO -Bindungskurven rechtwinkelige Hyperbeln ergeben also ohne den sonst obligaten Knick einer bei Null startenden O_2 - oder auch CO -Sättigung zu ergeben (s. S. 163/66 in „Respiration“). Dies ist aus den Haldane sehen Originalkurven zu ersehen (s. Abb 9). Das aber beruht (meiner Meinung nach) dass im Luft- O_2 -vorgesättigtem Blut kleine CO -Drucke im oberen und nicht im untersten Teil der Hb-Sättigungskurve eintreten. Dies geht aus den zuerst steil, dann flacher werdenden CO -Bindungskurven der Abb 9 hervor weil die ersten CO -Mengen im oberen Ende der Hb-Sättigungskurve noch keinen Konkurrenzkampf mit dem O_2 ausfechten müssen.

Würde aber das CO zuerst am untersten Punkte

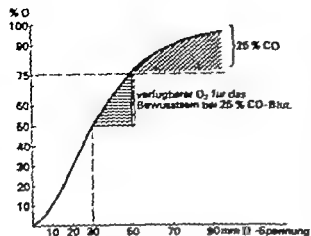


Abb. 8 Zeigt das zwar reduzierte aber immerhin noch genügende O₂-Produkt aus Menge und Spannung.

der O₂-Bindungskurve eindringen, dann müsste die CO-Nachsäuerungskurve auch die initiale Erschwerung aufweisen. In Analogie zur gewöhnlichen CO-Hb-Sättigungskurve. Letzteres ist ja durch Haldane tatsächlich bestätigt worden in Analogie zur initialen Erschwerung der O₂-Aufnahme eines blig O₂-frei gemachten Blutes (S. 165).

Haldane hat dieses nur so aufschlussreich erscheinende Ergebnis nicht für die menschliche CO-Vergiftung als Erklärung herangezogen, sonst hätte er auch zum Schluss kommen müssen, dass bei der CO-Vergiftung nicht nur die Menge, sondern auch die Endspannung des noch physiologisch bindungsfähigen O₂ im teilweise CO-belegten Blute verringert werden.

Für diese Auffassung, dass kleinste Mengen von CO das oberste Ende der O₂-Sättigungskurve beim lebenden Menschen belegen und deshalb praktisch subjektiv nicht empfunden werden, sprechen die Angaben im Prospect für das moderne CO-Oxymeter der Instrumentation Laboratory Inc. Lexington (Mass.) USA. Danach finden sie bei Bewohnern grosser Städte die Nicht-raucher und symptomlos Blut CO-Werte bis gegen 2% vor, bei harten Rauchern sogar bis gegen 10%.

Demnach muss man einem normalen Lungen eingeatmeten CO untersten Ende der Sättigungskurve drängen.

Man könnte dieser Kurve von der obersten

h	da
d	er
he	d
B	ku

(CO)-Ein
kurve

her also von der umgekehrten Seite her vorhalten sie sei rein spekulativ. Zur Überprüfung dieser Frage haben wir jedoch an 5 Mitgliedern einer unserer Jungfrauoch-Expeditionen am ersten Tage des Höhengaufenthaltes (3450 m) in ungerührter Liege Lage während 20 Minuten ein unreduziertes 4,5%iges CO-Luftgemisch atmen lassen (was einem reduzierten Werte von 2,88% CO entspricht) das während der ersten 10-12 Minuten im Gegensatz zu den Talversuchen ohne jegliche Änderung des subjektiven Befindens ertragen wurde. Die Blut-CO-Werte konnten dabei gesüßelt auf unsere zahlreichen Talversuche nur ungefähr 10-12% betragen haben. Erst nach dieser Zeit geschah eine leichte Pulsfrequenzerhöhung von 64 auf 69/Min. als die Versuche nach 20 Minuten abgebrochen wurden. Wenn wir nun aber in Liege Lage 6-8 tiefste Hyperventilationsatemzüge ausführen, so wurden alle 3 Versuchspersonen vorübergehend bewusstlos, genau so wie bei Tal-Hyperventilation bei 5-10%iger Blut-CO-Sättigung. Auch für diese Versuche wäre die Erklärung nach der Haldane'schen Vorstellung nicht möglich. Sie ist aber wiederum die, dass das CO von oben her die Bindung mit dem noch freien Hb startet und sich deshalb zunächst nicht merkbar auswirkt, weil ohnehin die obersten 10-15% des Hb auf Jungfrauoch-Höhe nicht durch den schwächeren O₂-Druck belegt werden, hier dringt

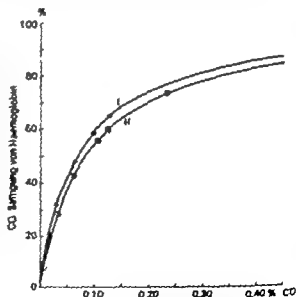


Abb. 9 Sättigungskurven von CO-Hämoglobin bei konstant 20.9 f. O₂ und 38° Temperatur. (Blut von J. S. H. Haldane, J. S. H. Haldane, J. S. H. Haldane, J. S. H. Haldane, J. S. H. Haldane).

das CO konkurrenzlos ins Hb ein. Da noch kaum O₂ verdrängt wurde, ändert sich nichts im subjektiven Bild.

Ganz ähnlich atmeten Douglas und Haldane im Tal ein Luftgemisch entsprechend einer Höhe von 5000 m. Sie machten die Beobachtung, dass eine CO-Vergiftung in diesem Zustand ohne subjektiv andere Symptome ertragen werden konnte, wenn die totale Blut-CO-Sättigung 23% nicht überschritt (auf S. 279 des Buches von Haldane). Für mich wiederum ein Hinweis für restliche Auffüllung der zu 2/3 mit O₂ vorgefüllten Erythrocyten durch CO, was für die O₂-Spannung keinerlei Abbruch bedeutet.

Bei Annahme der neuen Erklärung, nämlich der herabgesetzten O₂-Spannung und Menge eines menschlichen, vitalen, CO-teilbelegten Blutes ergeben sich nun auch geringere Differenzen beim Vergleich einer leichteren CO-Vergiftung mit der Höhenkrankheit. Haldane macht in seinem Buche „Respiration“ auf die erhebliche Ähnlichkeit beider Zustände aufmerksam. Diese werden jedoch noch besser erklärt, wenn in beiden Fällen eine herabgesetzte O₂-Spannung der Erythrocyten vorliegt. Immerhin bestehen jedoch zwischen den Auffassungen von Haldane und zahlreichen Eigenuntersuchungen (s. Fussnote S. 1) mit vielen Versuchspersonen doch noch gewisse Unterschiede. So tritt in der Höhe nach Haldane und nach zahlreichen Eigenuntersuchungen (16 Expeditionen mit je 10–14 Versuchspersonen je 14tägig im Forschungsinstitut Jungfraujoch (3450 m) während der Frühlingsferien 1942–1960) doch noch gewisse Unterschiede. So tritt in der Höhe bei der Ruheatmung infolge der plasmatisch bedingten Herabsetzung der O₂-Spannung eine mässige Hypertension auf, was im Gegensatz zur praktisch normalen CO-Ruheatmung zu einer respiratorischen Alkalose des ersten Höhenlagers führt, auf welche letzteres auch Haldane hinweist.

Hinzu kommt also bei der Höhenatmung oder auch der stärkeren Tal-Hyperventilation noch der spezifische Alkaloseschaden, sogar in Form einer Überatemungstetanie bei starker Hyperventilation oder in grossen Höhen, welcher bei der CO-Vergiftung in der Ruhe fehlt. Letztere führt im Gegenteil zur Acidose, die Höhenkrankheit zu respiratorischer Alkalose. Allen auch diese neue Darstellung ergibt keine definitive Lösung des Problems, weil nämlich erst zu 50% mit CO nachgesättigtes Lungenblut dann gar keinen O₂ mehr

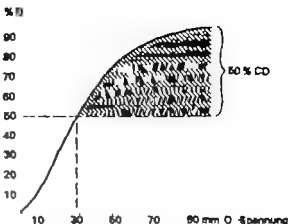


Abb. 10. Nach dieser Darstellung stünde dem Bewusstsein kein O₂ mehr zur Verfügung bei 50%iger Blut-CO-Sättigung, was schon im Liegen zum Coma führen sollte, was jedoch nicht der Fall ist.

für das Bewusstsein zur Verfügung hätte, da die ganze obere Hälfte der Hb-O₂-Bindungskurve in Analogie zur Abb. 10 bis zur 50%-O₂-Grenze hinab durch CO besetzt wäre. Dies aber würde einem völligen Bewusstseinsverlust gleichkommen. Da jedoch ein Mensch mit 50%iger Blut-CO-Belegung im Liegen noch durchaus nicht komatös ist, muss die Annahme gemacht werden, dass normalerweise der O₂ in der Lungenluft bis auf mindestens 20 mm Spannung abfallen kann, sofern der CO-Gehalt des Blutes nicht wie in der Höhe verringert wird, sondern konstant bleibt oder sogar (wegen erhaltender Atemgrösse) leicht ansteigt. Diese Annahme wird im folgenden Abschnitt experimentell begründet.

Durch Berücksichtigung dieser Faktoren wird in Abb. 11 nun ersichtlich, dass trotz Vorliegens einer 25%igen CO-Sättigung des Blutes *intra vitam* zwar noch ein recht erheblicher O₂-Vorrat zur Verfügung steht, dass jedoch bei kurzer Hypertension wegen des Bohreffektes (mit der Linksverschiebung der O₂-Bindungskurve) und der so schwerwiegenden Rechtsverschiebung der für das Bewusstsein verantwortlichen, minimal nötigen O₂-Spannung es zum Koma kommen muss. Die kleine obere O₂-Fläche der Abb. 11 ist eben unterschwellig für das Bewusstsein geworden. Als Vergleich seien die normalen O₂-Verhältnisse in einem Lungenblut in Abb. 12 dargestellt bei Innehaltung der CO₂-Spannung von 40 mmHg. Bei einem Atemhaltversuch oder gar in einem schweren Asthmaanfall kann der O₂ dank der

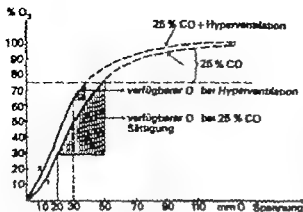


Abb 11 Die senkrecht schraffierte Fläche stellt das für das Bewusstsein verfügbare O_2 -Produkt in einem mit 25% CO-tesigenen Vitalblut dar. Die kleine horizontale schraffierte Fläche entspricht dem bei Hyperventilation resultierenden, zu Koma führenden zu kleinen O_2 -Betrag.

Acidose noch wesentlich tiefer im arteriellen Blut absinken. So erinnere ich mich an eine 50-jährige Asthmatikerin, welche nach einer in Lokalanästhesie ausgeführten Operation einer stenosierten Struma mit 2-stündiger Latenz in ein aller schwerstes Koma asthmaticum verfiel. Trotz aller vorher wirksamen Mittel blieb die Patientin während einer vollen Stunde tief bewusstlos im äußersten Cyanose. Die Rettung brachte dann eine i.v. Injektion einer Ampulle Asthmolylin, welche zu baldiger Normalisierung der Atmung führte. Aus der Ohnmacht erwacht verblieb nur eine ca. 3 Stunden lang dauernde Lähmung des

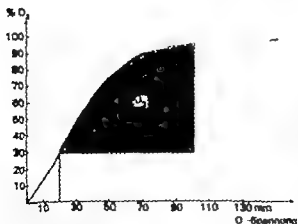


Abb 12 zeigt als Vergleich das Produkt aus Menge und Druck des im normalen Blute zur Verfügung stehenden O_2 für das Bewusstsein, bei normalem Blut pH.

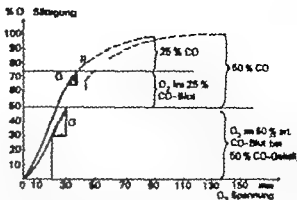


Abb 13 Kurve I Verhältnisse des intravitalen Blut- O_2 -Angebotes bei 50%iger Blut-CO-Sättigung. Die schraffierte Fläche stellt das im 50%igen CO-Blut noch für das Bewusstsein zur Verfügung stehende, zwar geringe Produkt aus O_2 -Druck und O_2 -Menge dar. Kurve II stellt die Verhältnisse dar bei 25%iger Blut-CO-Sättigung und kurzer Hyperventilation. Der O_2 -Vorrat ist zu unterschwellig geworden: Koma.

rechten Armes mit nachfolgender völliger körperlicher und geistiger Restitution!

Nun kann aber ein Mensch mit 50%iger CO-Blutsättigung im Liegen und bei Normalatmung den O_2 dank normaler CO_2 -Spannung in eben noch genügendem Masse ertragen. Erst durch Mithinwirkung der alkalischen bedingten Erschwerung der cerebralen O_2 -Verwertbarkeit gelingt es unser eingangs beschriebenes Hyperventilationskoma bei nur 25%iger CO-Belegung des menschlichen Blutes zu erklären (s. Abb 13).

Es soll deshalb noch näher auf den durch O_2 -Mangel oder Hyperventilation bedingten Alkaloseeffekt eingegangen werden.

Zu Wirkung der Alkalose auf das Gehirn

In der physiologischen Literatur ist allgemein bekannt, dass normale untrainierte Menschen in der Höhe oder im Unterdruck oder auch bei O_2 -Mangelatmung im Tal in grossen Räumen oder bei ständiger Absorption der ausgeatmeten CO_2 dann an die Grenze des Bewusstseins gelangen, wenn die O_2 -Spannung des arteriellen Blutes auf 30 mm (=50% O_2 -Blutsättigung) abgesunken ist. Nach Christensen u. Krogh fallen junge Leute bei Fliegererregungsprüfungen also dann zusammen, wenn die O_2 -Spannung ihrer Lungen unter 35 mmHg abfällt. Dies entspricht ungefähr 58% des Atmosphärendruckes (ref. bei Y. Henderson, 1941).

Hierbei ist die alveoläre CO_2 von ca 40 mm Spannung in der Regel auf Werte von ungefähr 20 mm gesunken als Folge der durch O_2 -Mangel bedingten Atemsteigerung. Es resultiert dadurch eine messbare Verschiebung des Blut-pH nach der alkalischen Seite in der Höhe oder im Unterdruck. Bei Pendelatmung im Tal in grösserem Raum mit fortlaufender CO_2 -Absorption konnte an der Grenze des eben noch Erträglichen ein 9.4%iger O_2 -Gehalt in der restierenden Luft durch Haldane festgestellt werden (S. 223). Im grossen Gegensatz hierzu konnte eine Pendelatmung mit nur 6 Litern Luft mit teilweiser CO_2 -Absorption der O_2 bis auf 4.6% herab erbracht werden. Dies ergibt deutlich den durch die Bohrkurven (O_2 -Bindung) von verschiedenen CO_2 -haltigen Luftgemischen nicht voll zu erklärenden Effekt der Alkalose, worauf auch Haldane auf S. 188 hingewiesen hat.

In ähnlicher Weise konnte auch Gellhorn zeigen, dass Versuchspersonen welche in einer Luft von 7% O_2 (=ca 50 mm O_2 -Spannung) oblag in kurzer Zeit bewusstlos werden, diesen O_2 -Mangel ohne weiteres ertragen können, wenn der Einströmungsluft 3% CO_2 zugefügt wurden. Diese Angaben konnten wir durch Untersuchungen unseres Schülers Leuppi anhand von 23 Versuchspersonen in einer Inauguraldissertation bestätigen.

Dieser so grosse Unterschied lässt sich meiner Meinung nach nicht durch die Bohrkurven erklären, denn der besseren Ausnützung des O_2 im saureren Blute bis auf tiefere Spannungswerte steht die durch die CO_2 -Zugabe gehemmte O_2 -Sättigung entgegen. Beide Faktoren halten sich also beim 7%igen O_2 -Atmungsversuch ungefähr die Waage, worauf auch Opritz aufmerksam gemacht hat.

Es soll daher näher auf den reinen alkalotisch bedingten Hyperventilationsschaden aufmerksam gemacht werden, der für das weitere Verständnis letzterem wichtig ist.

Ich habe meinen Schüler Neuwischwander 80 Versuche an gesunden, 22-30-jährigen Versuchspersonen ausführen lassen. Bei Zugabe von 4-6% CO_2 konnten Atmungsgemische mit nur 4-5% O_2 bei einem Barometerstand von 710 mmHg (entsprechend einer O_2 -Spannung von 28,5-35,5 mmHg in der Einatemluft) dank der nun möglichen, grossen Atmung ohne CO_2 -Verlust, also ohne Alkalose 3-4 Minuten lang störungsfrei ertragen werden. Hiernach waren die Atmungs-

sätze jeweils leer. Gleichzeitig ergaben Selenzellenwerte am histaminisierten Ohrfläppchen nach der Methode von Matthes bestimmt einen arteriellen Blut- O_2 -Wert von 20% entsprechend einer Spannung von 15 mm O_2 .

Eine willkürlich gleich gross gehaltene Atmung bei gleicher O_2 -Spannung aber ohne CO_2 führte dagegen durch die respiratorische Alkalose schon in 70 Sekunden zur Störungsschwelle mit Schriftfehlern und baldiger Bewusstlosigkeit.

Ein weiterer Hinweis für alkalotisch bedingte Störung der Gehirnfunktion wird durch die Hyperventilationstetanie geliefert.

Durch die alkalotische pH Verschiebung geschieht die für die Tetanie nötige Teil-Entkalkung des Calciums, worauf ich 1951 hingewiesen habe. Infolge dieser alkalotisch bedingten Verschiebung des Blut-pH am Abend des ersten Höhengaufenthaltes (z.B. Jungfraujoch 3450 m) treten nun auch bei Rubelage nach zahlreichen eigenen Versuchen bei gesunden jedoch nicht sonderlich an Höhengaufenthalte gewöhnten Versuchspersonen 2 T. heftige Kopfschmerzen auf. Diese sind alkalotisch bedingt und werden nach eigenen Versuchen durch Atmung von 3-5% CO_2 innerhalb von 20 Sek. zum vorübergehenden Verschwinden gebracht, nicht jedoch durch Atmung von reinem O_2 , sofern das Atemvolumen gleich gross gehalten ist, *ebende Luft imaug*.

Nun und aber Kopfschmerzen auch bei der CO_2 -Vergiftung zur Genüge bekannt. Jedoch gelang es uns im Gegensatz zum Höhenkopfschmerz nie durch CO_2 -Atmung (3-4%) diesen CO-kopfschmerz zu beheben. Er ist also nicht alkalotisch sondern acidotisch-hypoxisch bedingt und beruht auf perarteriellem Ödem der dilatierten Schädel- und Gehirngefässe mit langer Nachdauer (Wolff 1948, Lengggenhager 196...).

Im Gegensatz zur CO_2 -Atmung können nun (besonders bei jüngeren Menschen) infolge der ungewohnten Höhenalkalose selbst i. Rubelage richtige vegetative Gewitter entstehen mit Brechen und Schweissausbrüchen. Dagegen fehlen bei der CO_2 -Vergiftung nach zahlreichen eigenen Versuchen mit jüngeren liegenden Versuchspersonen diese vegetativen Gewitter.

Dagegen ist am Ende der CO_2 -Atmung nach eigenen Untersuchungen das Atemvolumen im Liegen nicht erhöht, sondern kann sogar am Schluss der 30-minütigen CO_2 -Atmung abnehmen, s. Abb. 4 und Abb. 1.

[illegible]

Abb. 14 Selbst im Atm. O_2 treten die für die Hyperventilation typischen Schriftstörungen auf. Sie sind also hier rein alkalotisch bedingt.

Eine Erklärung hierfür wird im Abschnitt „Warum ist das Ruhestoffvolumen bei CO-Atmung anfänglich nicht vergrößert“ gegeben.

Dass die akute hyperventilationsbedingte Alkalose (neben erschwerter O_2 -Abgabe aus dem Blute) noch einen Schaden an Gerüst darstellt, habe ich in fru eren Arbeiten beschrieben (1947 u. 1951). So zeigt B. Act. 14 schwerste alkalotische Störung einer 23-jährigen Versuchsperson mit forcierte Hyperventilation in unserer Überdruckkammer. m. O. bei 2 Atmosphären O_2 in H_2 mit der plasmatisch gelöste CO_2 an genügt zur Erhaltung des Bewusstseins beim Ausfall aller Erythrocyten. B. d. Belegung, was ja Haldane schon im Meeresschiff beweisen konnten. Die beschriebenen Sch

also alkalotisch und nicht hypoxisch bedingt gewesen sein.

Übrigens finden sich solche grotesken Schriftstörungen immer nur bei O_2 -Mangelattemptsversuchen nie jedoch bei rein hypoxischen CO_2 -Zuständen (s. Abb. 1).

In einer Arbeit habe ich (1951) auch nachgewiesen, dass psychische und tetanische Erscheinungen durch alkalotisch bedingte Teilentzerrungen des Calciums auftreten können. Dies ging besonders drastisch bei einem 25-jährigen Patienten hervor, welcher auf Grund eines nicht behandelten appendicetischen Abszesses durch Spontanperforation 2 hohe Dünnarmfisteln aufwies. Während zweier Tage stellten sich deliröse Schremlaffekte ein, die erst verschwanden, als der starke Blut-CA-Mangel erkannt und korrigiert worden war. Es erfolgte hernach schlussendlich völlige Heilung.

Auch dem Psychiater sind akute toxische Zustände bei Hypocalcämie bekannt. Schließlich sei auch daran erinnert, dass langsam i.v. verabfolgte 1 n NaOH beim markierten Tier (Kaninchen, Meerschweinchen) zum Tode führt, bevor es zur Bildung von Methämoglobin und Hämolyse schädlicher Ausmasse gekommen ist (eigene Versuche).

Banche verabreichte i v Alkalien bei Patienten mit Magen- und Duodenalgeschwüren und sah bei stärkerer Dosierung schockartige Zustände mit Zittern, Schwitzen und Kopfschmerzen.

Die Vorstellung, dass mit zunehmender respiratorischer Alkalose die cerebrale zellkatalytische Funktion bei Untrainierten im Schnelversuch leidet, geht wohl auch aus der Hyperventilationsohnmacht hervor.

Schon Haldane erwähnt die Hyperventilationsmacht, schreibt aber dazu deren Erklärung aus den Bohrkurven allein nicht zu geben weil er erklärt die le Andenken an Henderson Tiervacuole als zur Hauptsache durch den Blinddrucksturz bedingt. Diese Hyperventilationsversuche Hendersons waren aber an tracheotomierten Tieren manchmal ganz ausserordentlich stark forciert worden, wobei sogar die Expirationen unter nachstem Sog geziehen mussten, und sie dauerten zudem sehr lange, sodass schon die hierdurch bedingte Abkühlung der Tiere allein zum Erlischen der pontanen Atemarbeit und zu den starken Blutdruckstürzen führen konnte.

Au der folgenden Tabelle I unserer Eigenversuche ist aber ersichtlich, dass bei 4 gesunden

Tabelle 1 *Fallen von Blutdrucksturz bei starker Hyperventilation im Liegen trotz schon positiver Tetanie*

	Blutdruck vor Versuch (systolisch) (mmHg)	Blutdruck bei Auftreten Kribbels od. Tetanie bei starker Hyperventil (mmHg)	Dauer der Hyperventila- tion (Min.)
1. 24. j. ♂	125	120	11
2. 26. j. ♀	110	100	11
3. 22. j. ♀	105	110	4 1/2
4. 24. j. ♂	128	125	5
Durchschnitt	114,5	113,7	

Versuchspersonen bei kräftigster Hyperventilation in Lieflage bis zum Auftreten von starken Kribbelgefühlen und von Tetanie praktisch noch keine Blutdrucksenkung erfolgt, obwohl in diesem Stadium schon stärkste Schrittsstörungen sich geltend machen.

In der Literatur finden sich Angaben über Blutdruckabfall mit Hypozirkulation, durch respiratorische Alkalose bedingt, z.B. bei Henderson. Deshalb wiederholten wir an 3 weiteren, gesunden Versuchspersonen den Versuch mit der Modifikation dass in Lieflage die maximale Atemfrequenz und Tiefe angeführt wurden (1 Atemzug/Sekunde) bis zum Auftreten von Koma mit spontanem Atemstopp und z.T. klonischen Zuckungen was je nach der Versuchsperson in 1–3 1/2 Minuten der Fall war. In keinem dieser 3 Kurzversuche kam es zu einem Druckabfall im Gegenteil trat bei mir eine Blutdruckerhöhung von 120 mm (vor dem Versuch) auf 200 mmHg nach 3 Minuten und von 190 mm nach 3 1/2 Minuten beim Eintritt der Bewusstlosigkeit, auf. Nach Erwachen aus dem Koma wurde ich ungehalten weil ich glaubte man hätte die geplante Blutentnahme aus einer Cubitalvene zur Bestimmung des venösen Blut-pH beim Koma-Eintritt vergessen bis man mir den kleinen Wund erband an der Punktionsstelle zeigte. Mein Alter betrug 21 dieses Versuches 58 Jahre das meiner Mitarbeiter 76 und 77 Jahre. In allen 3 Fällen bestand nicht die geringste Schwierigkeit für die Venenpunktion da die Venen durchaus normal gefüllt blieben was für normale periphere Zirkulation spricht. Zur weiteren Prüfung der in der Literatur noch tretigen Frage der peripheren Blutzirkulation während starker respiratorischer Alkalose gingen wir folgendermaßen vor:

a) körpereigene Erythrocyten wurden diffus in

eine Ohrkapsel injiziert. Nach 2 Stunden wurde durch eine Selenzelle der Gewebs- O_2 in diesem Ohrkapsel bestimmt zunächst bei Ruheatmung, dann bei O_2 -Atemung ferner bei stärkster Hyperventilation in gewöhnlicher Luft bei O_2 -Mangelatmung und nach völligem Unterbruch der Blutzirkulation.

Die Abb. 15 zeigt dass während stärkster Hyperventilation der Gewebs- O_2 dieser Erythrocyten ebenso erhöht war wie bei Atmung von reinem O_2 , während er in der darauffolgenden Apnoephase stark und nach Unterbrechung der Blutzirkulation des Ohrkapsels durch schmale Klemme (oder durch Leertreiben der Gefäße des Ohrkapsels durch angepresste Glasscheibchen) maximal abfiel näheres siehe in der Originalarbeit von Lenggenger (1947).

Umgekehrt ergaben sich deutliche Unterschiede dieser extravasalen Erythrocyten- O_2 -Werte wenn anstelle gewöhnlicher Lufthyperventilation gegen nur 10% O_2 -haltiges Luftgemisch in gleicher Weise hyperventiliert wurde. Jetzt nahm der Gewebs- O_2 der extravasalen Erythrocyten von ungefähr 77 auf ungefähr 70 Skalenteile ab (s. Abb. 15a).

Nun darf man aber diesem Versuch nicht zu viel Bedeutung zusprechen, da CO_2 -verarmtes Hb viel leichter mit O_2 aufsättigen lässt. Um diesem Einwand zu begegnen, haben wir noch eine andere Versuchsanordnung gewählt.

b) Beweisender erflehen Indessen auch die Hyperventilationsversuche wenn in durch Jontophorese histaminisierte Arm- und Beinhaut Iodgoleukoblaquaddeln aus der gleichen Vorratspritze stammend gesetzt wurden und die Zerten bis zu deren Blaufärbungen verglichen wurden.

Während stärkster Hyperventilation waren die Färbungszerten kürzer als bei Ruheatmung, deut-

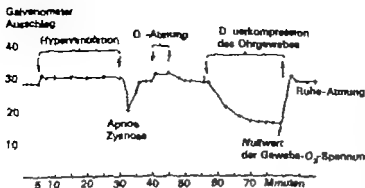


Abb 13 O_2 -Gehalt im histaminierten Ohrkläppchen-Gewebe vor und während starker Hyperventilation bei O_2 -Atmung und nach Zirkulationsunterbruch.

lich verlängert jedoch bei Hyperventilation von 10% und 84% O_2 . (Näheres siehe in der Originalarbeit Lengenbeger 1947)

Setzen wir diese stark forcierten Hyperventilationsversuche weiter fort (wobei bei einigen Versuchspersonen ständige Ernährungsmaßnahmen nötig waren) so trat wiederum kurze Bewusstlosigkeit auf.

Gegenüber dürfte die O_2 -Bestimmung am histaminierten Ohrkläppchen nach Matthes nicht zur Lösung der Frage des Gewebe- O_2 -Gehaltes bei Hyperventilation befähigt werden, da hierbei nur der O_2 -Gehalt der intravasalen Erythrocyten gemessen wird und nicht der Gewebe- O_2 -Gehalt.

Durch diese Versuche ist der Beweis geliefert, während stärkster hyperventilatorischer Alkalose die O_2 -Versorgung des Hautgewebes durchaus eine gute ist, was wegen der erhöhten Pulsfrequenz, dem praktisch normalen Blutdruck und der starksten gesteigerten Lungenbelüftung durchaus begründet erscheint.

Hingegen gibt dieser Versuch keine Antwort darüber, ob während dieser Hyperventilation das Hautgewebe normal oder vermindert atmet, indem

schließlich der O_2 im Hautgewebe auch ungenutzt sich verflüchtigen könnte (was jedoch mit Rücksicht auf das weniger spezifizierte Hautgewebe noch wahrscheinlich ist).

Zur Erklärung der Alkaloseohnmacht müsste man jedoch die in der Physiologie vertretene Ansicht einer mit zunehmender Alkalose einhergehenden cerebralen arteriellen Vasokonstriktion berücksichtigen (Kety u. Schmidt, 1946) während der periphere Blutdruck konstant bleibt (Kontos u. Mitarb.). Allerdings kommt diese Erklärung für die beschriebene, nach nur 8-10 tiefen Atemzügen auftretende Ohnmacht bei leichter CO_2 -Vergiftung sicher nicht in Betracht.

Im Gegensatz zu diesen Arbeiten ist andererseits nach Singh u. Mitarbeitern die cerebrale Durchblutung bei der Höhenkrankheit (also bei O_2 -Armut) gesteigert. Andererseits geschieht unter Hyperventilation nach Kety u. Schmidt (1948) eine Erhöhung des cerebralen Gefäßwiderstandes. Aber trotz der herabgesetzten Hirndurchblutung blieb die O_2 -Aufnahme im Gehirn normal, was das Vorhandensein einer wesentlich allgemeinen Hypoxiebeschädigung des Gehirns durch Hyperventilation widerlegt, für welche die Abnahme des O_2 -Verbrauches sonst ein Leitsymptom darstellt (Kety u. Schmidt, 1946).

Auch erwiesen sich längere mässige Hyperventilationsphasen an narkotisierten Menschen deren Bewusstseinzentrum obsolet ist durchaus nicht als schädlich. Sie Anästhesisten direkt gefordert, tieferen Acidose mit $NaCl$ (Tachirren, Quedes u. Gr. u. Parkers, etc.).

Übrigens tritt nach einer Überatmungstetanien nicht mehr in Erscheinung, doch tiefer M

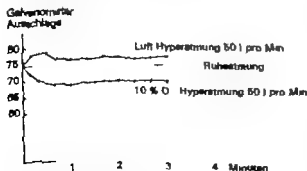


Abb 15 zeigt, dass schon in 10% O_2 die Erythrocyten des Ohrkläppchen-Gewebes bei H. per m. mg. von 10 l/min. deutlich geringeren O_2 -Gehalt aufweisen als bei gleichgroßer Luftatmung.

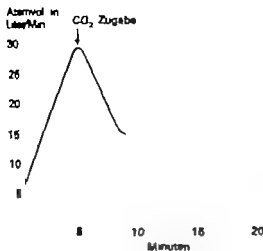


Abb. 16 Starke Hyperventilation beim Einatmen eines Luftgemisches von 7% O_2 und 93% N_2 . Bei Zugabe von 3% CO_2 Steilübersteigerung der Atmung auf ein Niveau von 30 Litern pro Minute. Durchschnitt von 7 Versuchspersonen.

dämpfender Effekt dieser Hyperventilation für das Bewusstsein darin, dass solche nur mit Lachgas und Relaxantien narkotisierte, überventilierte Patienten unserer Chirurgischen Klinik in eine tiefere Narkose zu bringen sind als ohne Hyperventilation, sodass keine zusätzliche Narkotika gebraucht werden müssen (Tachiren).

Die Frage einer möglichen cerebralen Hypocirculation im alkalotischen Gehirn des wachen Menschen musste also experimentell noch genauer erbracht, resp. bestätigt werden. Sollte sich eine solche bestätigen, so wäre dies eine zusätzliche Erklärung zum alkalotischen Gehirnschaden mit

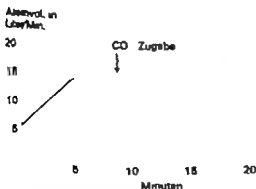


Abb. 16b Mittelmässige Atmung in einem Luftgemisch von 7% O_2 und 93% N_2 , bis auf 15 Liter pro Minute steigend. Bei Zugabe von 3% CO_2 bleibt die Atmung konstant. Durchschnitt von 9 Versuchspersonen.

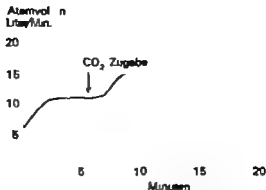


Abb. 16 Bei einem Luftgemisch von 7% O_2 und 93% N_2 sieben wir nur einen kleinen Anstieg bis auf 12 Liter pro Minute. Es folgt aber mit 3% CO_2 doch noch eine Steigerung der Atmung bis auf 18 Liter pro Minute. Durchschnitt von 7 Versuchspersonen.

der durch stärkste Hyperventilation bedingten Ohnmacht.

Durch Atmung O_2 -armer Luft können nach Untersuchungen meines Schülers Leuppi bei ungefährl. 1/3 der 3 untersuchten Studenten sehr starke Hyperventilationen ausgelöst werden. Dies wurde schon durch Haldane auf S. 184 seines Buches „Respiration“ beschrieben. Jedoch haben wir zuerst in einem Eigenversuch dann in einer Inauguraldissertation von Leuppi gefunden, dass bei solchen Versuchspersonen eine Zugabe von 3% CO_2 bei gleichbleibendem O_2 -Mangel der Einatemluft das Atemminutenvolumen sich paradoxerweise stark verkleinert (s. Abb. 16). Dies kann nicht durch den Bohreffekt erklärt werden, da ein anderer Teil der Versuchspersonen bei Atmung gleicher Gasgemische weder die sehr starke Hyperatmung aufweisen noch auf CO_2 -Zugabe die nur mittelstark vergrösserte Atmung steigern. Erst die restlichen Versuchspersonen zeigten in O_2 -armer Luft keine wesentliche Steigerung des Atemvolumens. Sie wurden einfach progressiv cyanotisch bis zum Präcoma. Zugabe von CO_2 in diesem Stadium führte zu grosser Atmung und damit zur Erholung (s. Abb. 16c).

Diese Beobachtung zeigt den zunächst erregenden Effekt des alkalotischen Blutes auf das Atemzentrum bei einem Teil der normalen Versuchspersonen. Auf eine in mittleren Höhen (3500 m) beim Menschen existierende Herabsetzung der Reizschwelle (also im Sinne einer besseren Ansprechbarkeit aller Reflexe) haben u. a. Grandjean

Tabelle 2 Tödliche Wirkung von i v verabreichter NaOH im Kaninchenversuch welche nicht durch die resultierende Hämolyse erklärt wird

Menge n/NaOH in ccm	i Inj Dauer	Hämolyse	Resultat
3 $\frac{1}{2}$	4	Stark	†
3,0	2 $\frac{1}{2}$	Stark	†
2 $\frac{1}{2}$	4	Stark	†
2,0	1	Stark	†
2,0	1	Stark	†
1,5	1 $\frac{1}{2}$	Mittelstark	†
1,5	1	Mittelstark	†
1,0	30'	Mittelstark	Überlebt
1,0	45'	Mittelstark	Überlebt
1,5	45'	Mittelstark	Überlebt
2,0	10'	Mittelstark	Überlebt
i n/NaOH			
8 cc	4	Stark	†
6 cc	2'	Stark	†
9 cc	12'	Stark	Überlebt

ferner auch Fleisch und v. Murralt 1944 hingewiesen. Dieser Erregungszustand kann bei weiterer Hyperatmung oder O_2 -Mangel jedoch ins Lähmungstadium übergehen.

Als Ausdruck eines cerebralen Alkaloseschadens fand ich ferner folgendes an mir und an solchen und Versuchspersonen die gleich mir bei Mangelatmung ein stark gesteigertes Ruholumen aufwiesen, wenn man in Liege Lage 3 tiefe Atemzüge Stickstoff atmet und den Atem bei der letzten N_2 -Inspiration anhält, so kann man kurz vor Einsetzen des imperativen Atemreizes Zeichen vorübergehender Hypoxie beobachten entweder Flimmern oder Verdüsterung des Gesichtsfeldes oder klonische Zuckungen der Kopf- oder Gesichtsmuskulatur. Alle diese Symptome verschwinden wieder kurz vor Einsetzen des gewöhnlichen Atemreizes weil der Alkaloseschaden trotz der progressiven Hypoxie durch die CO_2 -Anreicherung kompensiert wird.

Ein direkter Alkaloseschaden geht nach eigenen Versuchen auch daraus hervor dass leicht oder mäßig starke Kaninchen bei einer Injektion von ca. 1,5–3 ccm n/Natronlauge sterben. Die hierdurch auftretende Teilhämolyse ist zu schwach um den Exitus zu erklären da Injektionen von hämolyisiertem Blut mit der gleichen Schlusskonzentration ohne schädliche Wirkung ablaufen (Tab. 2 sind die genaueren Verhältnisse dargestellt).

Nun kann sich aber der Organismus mit der Zeit auch an die sonst ungewohnte Alkalose gewöhnen.

In diese Richtung weisen Untersuchungen von Cameron der die Höhenanpassung nicht eigentlich in der Wiederherstellung eines normalen Gewebe- O_2 -Druckes sieht sondern vielmehr in der Fähigkeit des Körpers den Gasaustausch trotz erniedrigten O_2 -Druckes aufrecht zu erhalten.

Auf die Bedeutung der β -Phosphoglycerate für die O_2 -Sättigungskurve des Blutes wird später kurz eingegangen.

Umgekehrt findet sich auch eine vorübergehende Störung vor bei Menschen, die immer in der Höhe lebten (Anden) und dann ins Tal abstiegen.

Mit der Zeit tritt eine Gewöhnung auch bei (jüngeren) Tieren an die Höhenhypoxie auf welche sich selbst nach längerem Talaufenthalt nicht verliert ohne dass Verruz (1960) dafür eine Erklärung geben konnte. Dies scheint für eine zellkatalytische Verbesserung der O_2 -Verwertung im hochaklimatisierten Gehirn zu sprechen denn dies kann anhand der Hb- O_2 -Bindungskurven nicht erklärt werden. So konnten wir durch den hochgewohnten Alpinisten André Roch (der an einer Himalaya-Expedition teilgenommen hatte) im Forschungsinstitut Jungfraujoch ein Atmungs-gemisch von 7% O_2 bei einem Barometerdruck von nur 495 mmHg atmen lassen was einem O_2 -Partialdruck von nur 33 mmHg in der Einatemungs-luft entspricht! Dieses Gemisch konnte bis zum Ende des 200 Liter fassenden Atmungs-sackes geatmet werden ohne dass es zu merklichen Störungen kam wohl aber wurden grosse langsame und tiefe Atemzüge von je 3 Litern geatmet. Diese Versuchsperson war also an die Alkalose so gewöhnt dass deren cerebrale Schäden sich nicht mehr (oder erst bei stärkerem Grade) einstellten.

Im Gegensatz hierzu wurden alle 3 sich zu diesem Versuch hingebenden Versuchspersonen unserer Gruppe innerhalb von 1–1 $\frac{1}{2}$ M. bewusstlos.

Schon Barcroft und auch Hurtado (1928) fanden, dass langjährige Höhenbewohner keineswegs eine höhere O_2 -Sättigung des Blutes aufweisen und betonten dass gute Höhenverträglichkeit nicht an hohe O_2 -Sättigung des Blutes gebunden sei.

Desgleichen berichtete Campbell 1927 anhand von wochenlangen Tierversuchen mit Atmung O_2 -armer Luft, dass die Gewöhnung nicht in Erreichung normaler Gewebe- O_2 -Drucke sondern vielmehr auf der Fähigkeit beruhte trotz nied-

nger O_2 -Spannungen in den Geweben den Gasaustausch aufrecht zu erhalten

Im gleichen Sinne sprechen auch die Untersuchungen von Lahiri u. Mitarbeitern (1967) und Lahiri (1968) ferner alle "blue babies" mit angeborenem Herzfehler und der beschriebene asphyktische Asthmaanfall mit dem stündigen höchst cyanotischen Komazustand und nachfolgender völliger Resitution

Nach allen diesen Besprechungen über die respiratorische Alkalose scheint also das Gehirn das hauptleitende Organ zu sein. Es ist auch das wertvollste empfindlichste Organ gegen O_2 -Mangel.

Ein Gegenstück zu einer durch akute Alkalose bedingten funktionellen Hirnschädigung findet sich nun auch in einem durch Schädigung der Gehirngefäße bedingten Hirnschaden vor welcher zu Störungen der metabolischen Oxydationsprozesse durch die Lactacidose bedingt ist (Ingvar u. Lassen) Erhebliche derartige Veränderungen können innerhalb von Minuten eintreten. Die saure pH-Verschiebung führt dabei zu einer massiven und langanhaltenden Vasodilatation die als Vasoparalyse bezeichnet wird Diese kann nach Ingvar u. Lassen länger andauern als die Periode der Anoxie/Hypoxie selbst, auch wenn die Durchströmung wieder hergestellt ist sodass der Durchfluss die Bedürfnisse des Gewebsstoffwechsels längere Zeit überschreitet welchen Zustand man als Luxusperfusion bezeichnet.

Es ist diese stark acidotisch bedingte Schädigung der Hirnzellen eine Art Gegenstück zu akuter alkalotisch u. Hirnschädigung Ein acidotisches Gehirn arbeitet ebenfalls nur noch bei hoher O_2 -Angebot

Nun interessiert uns die Frage ob nicht mit neuesten Geräten auch eine klinisch zu erwartende Herabsetzung der O_2 -Spannung für die Endaufklärung des normalen aber an CO_2 verarmten menschlichen Blutes festzustellen wäre Die bisher üblichen O_2 -Bestimmungen im Slyke Apparat scheinen für die letzten Sättigungsgrade hierfür nicht geeignet wegen der zu kleinen für die Analyse zu verwendenden Blutmengen.

Wir bedienen uns daher wiederum des vorstehend beschriebenen spektrometrischen CO -Oxymeters, welches auf 1% genau die O und CO -Sättigung des Blutes bei 37°C angibt (s. Abschnitt „Zur Kritik an der Haldane'schen Aufassung“).

Versuchsbedingungen für die O_2 -Sättigungskurven bei verschiedenen CO_2 -Spannungen
on 40 0 10 0 mm Spannung CO

1. Verwendet wurde menschliches, heparinisiertes Frischblut mit N reduziert (früher bereits beschrieben)

2. Gemische von je 150 l mit verschiedenen O_2 und CO_2 -Spannungen werden in einem 200 l fassenden Gummisack stets frisch hergestellt und mit der auf S. 15 beschriebenen Formel errechnet Die Ergänzung der CO_2 und O_2 -Mengen auf 150 l geschieht jeweils mit reinem O_2 freiem N

3. In Abb. 6 ist das genaue Versuchsschema gezeigt 4 cc Blut werden durch Hahn A in das mit dem jeweiligen Gasgemisch vorgefüllte System eingefüllt. 10 Minuten lang wird abwechselungsweise das Blut kräftig in dem Kolben geschüttelt und nach je ca. 30 Sekunden wieder neues Gasgemisch mittel Wasserstrahlpumpe durchgesogen. Für die Blutproben-Entnahme gelten die gleichen, früher angegebenen Kriterien.

Die Untersuchungen meines Dissertanten Spycher mit diesem Gerät ergaben nun die aufschlussreiche Tatsache dass mit zunehmender CO_2 -Verarmung die O_2 -Bindungskurven des menschlichen Blutes steiler verlaufen unter progressiver Linksverschiebung auch unter Endsättigung Dies heisst also dass die O_2 -Spannung im alkalotischen Erythrocyten (bei gleichem O_2 -Druck der Sättigungsluft) kleiner ist als im normalen CO_2 -haltigen Blut weil der O_2 leichter aufgenommen wird Die Abb. 17 zeigt diese Ergebnisse meines Schülers Spycher In Abb. 17 sind diese Verhältnisse bildlich dargestellt. Es scheint aber nicht ausgeschlossen, dass bei stärkerer Hyperventilation die O_2 -Verwertungsschwelle für das Bewusstsein sogar noch auf 40 mm Spannung heraufgetrieben wird was allerdings in der Abb. 17a nicht berücksichtigt wurde, wohl aber in der Abb. 18

Diese progressive Herabsetzung der O_2 -Spannung in einem zunehmend hyperventilatorischen Blute zusammen mit der alkalotischen Erregung der cerebralen O_2 -Verwertung (mindestens im Bewusstseinzentrum) dürfte also die Hauptrolle spielen für die Erklärung der Hyperventilationsohnmacht. (Dieser Alkaloseschaden wird durch allfällige cerebrale Hypozirkulation noch verstärkt.)

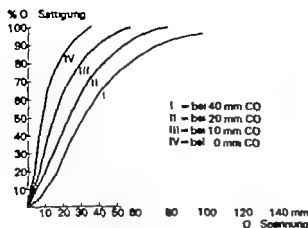


Abb 17 Neue O_2 -Sättigungskurven des menschlichen Blutes bei abnehmendem CO_2 -Gehalt, bestimmt mit dem modernen CO_2 -Oxymeter** bei $38^\circ C$

Der Begriff eines lokalisierten, cerebralen Bewusstseinszentrums ist nicht neu. Brexlaue hat schon vor 40 Jahren an in Lokalanästhesie operierten Hunden nachgewiesen, dass im basalen Hirnstammgebiet auf kleiner Fläche durch leichteres Beklopfen Bewusstlosigkeit auftreten kann, nicht aber von anderen Hirnteilen aus.

1945 habe ich anlässlich einer in Lokalanästhesie ausgeführten Kleinhirn-Tumor-Entfernung beim Menschen ein solches Bewusstseinszentrum in traglicher Weise bestätigen können. Nach Entfernung des ganzen rechten Kleinhirns blutete es hartnäckig aus einer kleinen Stelle der vorderen Medulla oblongata, sodass schließlich ein milder und ganz kurzer Elektrokoagulationsstoß auf diese Stelle eingeführt wurde. Die in der Abbildung dargestellte Elektrode lag unter dem

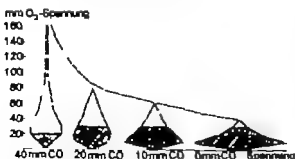


Abb 17 Bildliche Darstellung (der Abb 17) auf der erleichterten O_2 -Aufnahme in progressiv CO_2 -ärmeren Erythrocyten. Daraus gehen der zunehmende O_2 -Spannungsverlust und die abnehmende O_2 -Menge für das Bewusstsein hervor

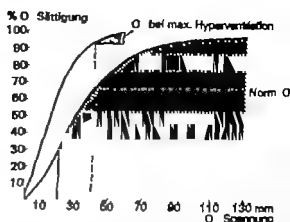


Abb 18 Die obere, kleinschriftliche Fläche zeigt die für das Bewusstsein unterschwellige O_2 -Menge bei stürkster Hyperventilation durch alkalotische Verschiebung der lokal-cerebralen O_2 -Verwertungsschwelle von 20 auf 40 mm Spannung (punktierter auch rechte Linie). Die untere schraffierte Fläche entspricht dem O_2 -Angebot bei normalem Blut.

Gestirbt dieser 32-jährigen, erblindeten Patientin im Moment der kurzen Koagulation erlosch schlagartig das Bewusstsein dieser vorher immer ansprechbaren Patientin für ca 1 Minute. Nach Erholung musste leider eine zweite in der hinteren Post-Gegend gelegene hartnäckige Blut-sickerstelle endlich auch durch kurzen Elektrokoagulationsstoß gestillt werden. Jetzt erloschen leider definitiv Bewusstsein, Atmung und Herzaktion. Die beiden Stellen sind in Autopsiepräparat der Abb 19 bezeichnet. Alle proximal dieser Stellen liegenden Gehirnteile waren vom Stromstoß verschont, damit ist ein lokalisiertes Bewusstseinszentrum auch beim Menschen erwiesen.

Die Tatsache, dass auch schwere Gehirnverletzungen durchaus nicht immer zu Bewusstlosigkeit führen müssen, haben wir öfters beobachtet. So brachte ein Patient, welchem ein Lastwagenrad langsam über den rechten Stirnpol gefahren war, bei Bewusstsein die aus der offenen Schädelfraktur in den Hut ausgepressten Stirnhirnmassen mit ins Spital und zeigte sie uns!

Auch haben wir Hunderdurchschüsse durch kleinkalibrige Revolvergeschosse erlebt ohne Bewusstseinsverluste, wenn die Kugeln z.B. fronto-frontal verließen, gelegentlich sogar mit inneren Reflexionen.

Auch quere Hinterhauptabtrennung durch Projektilverletzung führte nach Spatz nicht zu Bewusstseinsverlust. Es bedarf zu letzterem eben der

Druckwellenfortpflanzung in dem hinteren Hirnstamm, wo das Bewusstseinzentrum liegt.

Zusammenfassung über die Alkaloseschäden

Nach dem Besprochenen setzt sich also ein Hyperventilations Alkaloseschaden aus folgendem zusammen:

1 Erschwerte O_2 -Angabe aus dem alkalotischen Blute wegen stärkerer Bindung an das Hb selbst in den obersten Sättigungsgraden.

2 Hierdurch schädliche Herabsetzung der für das Bewusstsein so wichtigen O_2 -Spannung.

3 Gleichzeitig Herabsetzung der für ein alkalotisches Bewusstseinzentrum eben noch genügenden minimalen O_2 -Spannung.

4 Alkalotisch bedingter spezifischer Gehirnschaden unabhängig vom O_2 -Mangel (deshalb auch in der O_2 -Überdruckkammer auslösbar).

5 Teilentkalkung des Calciums durch die Hyperventilationsalkalose was u. a. zu cerebral bedingter Tetanie führt

6 Altfällige alkalotisch bedingte cerebrale Hypozirkulation.

Alle diese Faktoren summieren sich zum Bilde des schweren Hyperventilationsschadens.

Kombination von Alkalose und CO

Kommt nun zu den besprochenen Wirkungen der Alkalose mit der Erschwerung der cerebralen O_2 -Verwertung und der gleichzeitig alkalotisch bedingten Herabsetzung der Blut- O_2 -Spannung noch eine Teilbelegung des Blutes durch CO so gesellen sich also noch mehr 2 schädliche Faktoren dazu, die durch CO bedingte Verlingerung der O_2 -Menge und damit deren Spannung.

Unsere anfängliche Vorstellung über den Grund der raschen Bewusstlosigkeit durch Hyperventilation, beim Vorliegen einer CO-Teilbelegung des Blutes war die Annahme, es könnte sich der Bohrereffekt in einem solchen CO-Blute stärker auswirken. Doch ward dies durch die Untersuchungen von Haldane nicht bestätigt.

Die Blut-CO-Sättigung ward bei progressiver CO_2 -Verarmung im gleichen Verhältnis vermindert wie im normalen Blut (S. 165 Haldane)

Im Gegensatz zu Laborversuchen mit O_2 -Bindungsversuchen eines CO-teilorgesättigten Blutes geschieht also bei der menschlichen CO-Vergiftung das Umgekehrte: das Lungenblut das zunächst nahezu O_2 -gesättigt ist kommt progressiv mit einschleichendem CO in Berührung. Letzteres wird sich also am oberen Ende der



Abb 19 1 Stelle der Medulla oblongata, von wo aus durch schwächste Electrocoagulation Atmung u. Bewusstsein schlagartig ausgelöscht wurden. 2 Stelle in hinterer Pons, wo nach schwächster Electrocoagulation Koma u. Herzstillstand ausgelöst wurden. 3 Schnittfläche des 1. Hirschenkels. 4 Verblühtgegend. 5 linke Kleinhirn-Hemisphäre. 6 breit offener 4. Ventrikel. 7 Schnittfläche des Halsmarkes.

Hb- O_2 -Bindungskurve bei progressiv binden lassen, weil dort die geringste O_2 -Affinität herrscht, und damit der CO-Bindung zuerst kein Hindernis entgegen gebracht wird.

Neue Erklärung der Hyperventilationsohnmacht bei CO-Vergiftung

Nach dieser Besprechung des Kombinations Schadens von CO-Wirkung und Alkalose können nun die Verhältnisse beim Hyperventilationskoma endlich befriedigend erklärt werden.

Betrachten wir Blut einer nicht trainierten Versuchsperson, welches zu 25% durch CO nachbelegt wird

In Ruhe kann dieser verarmte O_2 bei normalem pH bis hinunter zu 20 mm O_2 -Spannung dem Gehirn zur Verfügung stehen. Kommt aber eine CO_2 -Verarmung infolge kurzer maximaler Hyperventilation dazu so wird wegen dieser Alkalose die für das Bewusstsein nötige minimale O_2 -Spannung auf mindestens 30 mmHg hinauf gedrückt wie wir das von der Höhenkrankheit wissen, wo wegen des gesteigerten Atemvolumens es schon bei einer 30 mm O_2 -Spannung zur Bewusstlosigkeit kommt. Dazu kommt nun also die hyperventilationsbedingte Linksverschiebung der O_2 -Bindungskurve welche nicht nur zur Herabsetzung der O_2 -Spannung, sondern gleichzeitig noch zur alkalotisch bedingten Verringerung der für das Bewusstsein nötigen O_2 -Menge führt.

1 Abb 11 sind die noch erheblichen O_2 -Produkte aus Menge mal Spannung in einem 25%

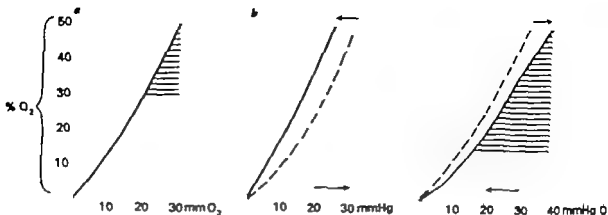


Abb 20 In *a* *b* und *c* handelt es sich um Blut, dessen oberste 50% durch CO beladen sind. (*a*) Genügend O_2 für den Bewusstseinszustand im Liegen (*b*) Bei kurzer Hyperventilation ist kein O_2 für das Bewusstsein mehr vorhanden. Koma. $CO_2=20$ mm bedingt eine Linksverlagerung der O_2 -Bindungskurve mit gleichzeitiger Rechtsverschiebung (also Erschwerung) der ce-

reinen O_2 -Verwertung. (*c*) Bei CO_2 -Atmung (80 mm CO_2) starke Vergrößerung des verfügbaren O_2 infolge Rechtsverschiebung der O_2 -Bindungskurve. Die unterbrochenen Linien stellen die normale O_2 -Bindung in normalen Blut dar, dessen oberste 50% durch CO -Nachsättigung belegt sind bei einer normalen CO_2 -Spannung von 40 mm.

CO -Blut für das Bewusstsein dargestellt im Gegensatz zum unterschwelligen O_2 -Produkt bei kurzer Hyperventilation, was zum Koma führt. Die Abb 12 entspricht den normalen Verhältnissen.

In Abb 13 ist ersichtlich, warum ein Mensch 50%iger CO -Blutsättigung (Feld 1) besser ist als bei 25%iger CO -Sättigung mit Hyperventilation. Würde jedoch ein Mensch mit 50%iger CO -Blutsättigung nur kurz hyperventilieren, so ergäbe sich überhaupt kein für das Bewusstsein zur Verfügung stehender O_2 -Vorrat mehr.

Die folgende Art der Darstellung eignet sich noch besser zur Wiedergabe der O_2 -Spannungsverminderung eines normalen Lungenblutes durch nachträglichen 25%igen CO -Besatz: In einem 5% durch CO befallenen Lungenblut hört die O_2 -Bindungskurve abrupt bei 75% auf in einem 50% CO -Blut bei 50%. Von diesen O_2 -Endsättigungspunkten steigen die CO -Kurven nahezu senkrecht an, da schon unter 1 mm CO -Spannung eine totale Blutsättigung erreicht wird. Dies wird durch die Abb 20 dargestellt.

Nun beginnt also die O_2 -Nachsättigung eines zu 50% durch CO vorgefüllten Blutes schon bei 1 mm O_2 -Spannung. Der O_2 -Nachsättigung und also die sonst für eine 50%ige Blutsättigung nötigen 30 mm Spannung durch die 50%ige CO -Vorsättigung abgenommen worden. Damit ist auch der O_2 -Endsättigungsdruck erniedrigt worden. Umgekehrt prüft sich auch das CO auf ein zu 50% durch O_2 vorgefülltes Blut schon unter

halb 1 mm CO -Spannung ab. Es droht dabei also zunächst wiederum an der untersten, noch nicht besetzten Hb-Stelle ein und erfüllt also von der Mitte der Sättigungskurve an aufwärts den Rest der Erythrocyten. Dies muss man aus den Haldane'schen Kurven, die sich bei zunehmenden CO -Drücken auf ein O_2 -vorgesättigtes Blut ergeben (S 166 Abb 9) schließen.

Nochmals zu Gegenüberstellung von CO und Höhenatmung

Haldane macht mit Recht auf eine gewisse Ähnlichkeit zwischen Bergkrankheit und CO -Vergiftung aufmerksam (S 240) obwohl er bei der CO -Vergiftung eine normale O_2 -Spannung im Blute annimmt. Nach neuer Auffassung ist dieser Vergleich noch zutreffender da in beiden Fällen die O_2 -Spannung herabgesetzt ist. Nur findet sich die Höhenatmung wegen des vergrößerten Ruhetatemvolumens mit einer respiratorischen Alkalose vergesellschaftet, die CO -Vergiftung jedoch mit einer Acidose.

Die Alkalose ist nach Haldane nicht nur am ersten Höhenstage sondern auch nach der Akklimatisierung nachgewiesen worden, (S 107 seines Buches „Respiration“). Wir selbst haben eine alkalotische Blut-pH-Verschiebung in 3 Höhenexpeditionen auf Jungfraujoch mit total 26 Versuchspersonen von 7,36 auf 7,49 während der jeweils ganzen 14tägigen Dauer bestätigen können (un-terhand des elektrischen Tena-Messgerätes). Die

gleiche alkalotische pH Verschiebung wiesen auch 2 dauernd auf Jungfrauojoch Angestellte auf

Wohl geschehen schon am 2. Höhestage durch Einströmen von Milchsäure aber auch besonders durch 2,3-Phosphoglycerat im Blut und in die Erythrocyten (nach Finch und Leaflet, und auch nach Benesch et al.) nach rechts verschobene O_2 -Bindungskurven der Erythrocyten. Diese Vorgänge spielen jedoch aus zellulären Gründen bei der akuten CO -Vergiftung noch keine Rolle

Diese Alkalose bedingt nun für Untrainierte die Erschwerung der cerebralen O_2 -Verwertung für das Bewusstsein welche Funktion also nur noch unter einem höheren O_2 -Angebot erhalten bleibt. Hierfür sorgt die vergrösserte Atmung. Wegen dieser Höhenalkalose wurden nun alle 5 eigenen Versuchspersonen auf Jungfrauojoch (3450 m) schon bei einem Blut- CO -Gehalt von nur 15% nach ca 8 tiefsten Hyperventilationsatemzügen komatös, während es im Tale bei den gleichen Versuchspersonen erst bei 25% CO -Blutgehalt zum Bewusstseinsverlust gekommen war

Bei der Höhenatmung jedoch wirkt sich die Alkalose zunächst deshalb nicht in deletärem Masse aus weil durch das vergrösserte Atemvolumen immer eine bessere O_2 -Sättigung des Blutes geschieht. Hierbei halten sich, wenigstens in nicht zu grossen Höhen, der schmälernde Bohreffekt einerseits und die bessere O_2 -Sättigung anderseits zunächst die Waage ganz anders bei der CO -Vergiftung. Hier besteht eine progressive hypoxische Acidose weil das Atemzentrum allmählich erfährt, trotz der zunehmenden Abblaufung hypoxischer intermediärer Stoffwechselstufen.

Die Acidose jedoch gesteuert dem Gehirn die bessere O_2 -Ausnützung des Blutes bis auf Werte sogar zu 20 mm O_2 -Spannung hinab. Dazu kommt zu bei schweren acidotischen Graden die CO -Vergiftung die Sa v -bedingte Rechtsverschiebung der O_2 -Bindung als einer höheren O_2 -Spannung diese Blut bei gleichbleibendem Luftdruck gleichkommt. Ohne das hierdurch mehr O_2 ins CO -haltige Blut auf genommen werden kann ist also dennoch die O_2 -Spannung angestiegen

Dies gibt meines Erachtens eine neue Erklärung zu Haldane Feststellung, dass bei CO -vergifteten Mäusen die ersten Symptome durchaus nicht verstärkt, sondern eher gemildert werden, wenn diese

Tiere menschliche Expirationsluft bei künstlich gleichbleibendem CO -Gehalt zu atmen bekommen, obschon nun eine geringere O_2 -Spannung in der Atemluft vorliegt. Haldane's Erklärung hierfür ist nämlich die Hebung des Blutdruckes durch bessere Herzaktion unter der CO_2 -Wirkung.

Nach der neuen Erklärung jedoch verringert die Einatmung von Expirationsluft die O_2 -Sättigung in CO -teilladeter Lungenluft keineswegs, da infolge der CO -Beladung immer noch genügend O_2 -Mengen vorliegen, um das O_2 -Manko dieses CO -Blutes völlig auszugleichen. Dagegen nimmt die O_2 -Spannung nun wegen der acidotischen Rechtsverschiebung der O_2 -Bindungskurve zu. Dies mag aus Abb 20 hervorgehen.

Unsere CO -Versuche auf Jungfrauojoch (3450 m) sind ein guter Beweis für die neue Auffassung des ersten CO -Eintrittes ins Lungenblut „von oben her“. Deshalb können ja in der Höhe (3450 m) die ersten 10% der Blut- CO -Sättigung in Ruhelage vollständig symptomlos ertragen werden, da zuerst nur die ohnehin leeren obersten Partien der Hb-Sättigungskurve CO -nachgefüllt werden. Nach Haldane's Vorstellung wäre gleich von Anfang der CO -Atmung an mit einer progressiven Reduktion des verwertbaren O zu rechnen was zu einem bald auftretenden Pulsanstieg führen müsste welchen wir jedoch in der Höhe bei nur 10-12% Blut- CO -Sättigung nicht feststellen konnten

Vergleich der Gefährlichkeit von CO -Vergiftung und Höhenatmung

Ausgehend von der Ansicht, nicht nur die physikalische, sondern auch die biologische O_2 -Spannung im CO -Blute sei im Tieflande eine normale vertritt Haldane die Ansicht es sei eine CO -Vergiftung nicht so gefährlich wie reiner O_2 -Mangel bei Atmung O_2 -armer Luft in grossen Räumen oder in der Höhe wo die tödliche Katakastroph häufiger beobachtet werde. Dieser Auffassung möchte ich etwas einschränkend entgegenhalten dass auch längere hypoxische Zustände beim Atmen O_2 -armer Gemische durchaus nicht immer deletär sein müssen. So konnte ich als 38-jähriger 1/2 Stunden in einer Unterkammer für Fiebererregungsprüfungen bei einem Unterdruck entsprechend einer Meereshöhe von 9000 m ohne O_2 -Atmung nur mit gelegentlicher CO_2 -Zufuhr (zur Behebung der sonst periodisch auf

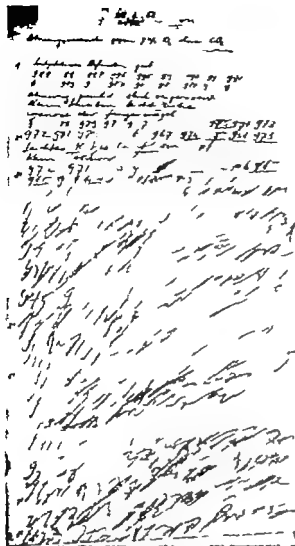


Abb 21 Über 15 Minuten anhaltende stärkste hypoxisch-alkalische Schriftstörungen einer 25j. ♂ Versuchsperson bei Atmung von 7% O₂. (Es wurde noch ein 2. Blatt unleserlich verfertigt)

tretenden starken Überatemungstetanie mit Schriftstörungen) sitzend verweilen und dauernd gute Schriftproben ausführen und Rechenaufgaben lösen, wie ich das 1942 beschrieben habe.

Dr F von Tavel der die Schweizerische Militär Unterdruckkammer für Fliegererregungsprüfungen konstruiert hat und dem ich obigen Eigenversuch verdanke erzählte mir dass er einmal in der Unterdruckkammer sitzend bewusstlos zusammenbrach und gemäss der Uhr in seinem Protokoll während 20 Minuten am Boden liegend im Koma verbarnte um dan bei gleichbleibendem Unterdruck sich sponta wiederum so weit

zu erholen dass er bewusst zur O₂-Atmung schreiten konnte

In Abb 21 sind recht langdauernde Schriftstörungen stärkster Art abgebildet, herrührend von einer 23-jährigen Versuchsperson, die in halb sitzender Lage über 19 Minuten bis zur völligen Leerung des Atmungsreservoirs ein Luftgemisch von nur 7% O₂ entsprechend einer Spannung von ca 47 mmHg (Barometerstand von 710 mmHg) atmete dauernd völlig unleserliche Zeichen kritzelte und nach dem Versuch keinerlei Störungen aufwies. (Leider sind die beiden folgenden Seiten dieser interessanten Schriftprobe beim Umzug unserer Klinik in ein neues Gebäude verloren gegangen)

Auch die klassisch gewordene Ballonhöhenfahrt von Sivel Tissandier und Spinelli 1875 (Haldane „Respiration“ S 319) welche zwar auf über 8000 m Meereshöhe zu 2 Todesopfern führte ergab immerhin einen die lange Bewusstlosigkeit Überlebenden mit keinerlei Nachstörungen.

Bedenkt man ferner dass die beiden anderen Ballonfahrer in sitzender Stellung im Ballonkorb bewusstlos zusammenbrachen, so ist gut vorstellbar dass wegen der resultierenden Kopfhängelage es zu einer Erstickung kommen konnte so wie auch nach längerer Narkose ein Patient in Rückenlage durch Zungenaspiration ersticken könnte wenn nicht der Unterleifer durch eine Hilfsperson am Zurückziehen verhindert würde. In der Armenunterdruckkammer konnte ich selbst einige Male miterleben, dass junge Fliegeraspiranten im Unterdruck entsprechend einer Höhe von nur 4500-5000 m ganz plötzlich aus ihrer sitzenden Stellung heraus bewusstlos und mit Krämpfen auf den Tisch hingefallen wären hätte die O₂-atmende Begleitperson sie nicht im letzten Moment gehalten und O₂-Atmung eingeschaltet. Je nach der Körperlage eines solchen hypoxischen Menschen könnte er nun, sich selbst überlassen durch Zungenaspiration ganz ersticken.

Im gleichen Sinne spricht eine UPI-Meldung aus Madrid vom 4.6.69 wonach der 22-jährige Armando Socarras Ramirez als blinder Passagier versteckt im Fahrwerkschacht einer DC 8 den fast 9stündigen Flug von Havanna nach Madrid bei einer Flughöhe von 9000 m ohne Druckkabine in bewusstlosem Zustande überlebte (Ref im „Der Bund“ Bern am 5.6.69)

Diese wenigen Beispiele sollten nur zeigen, dass unter Umständen auch starker O₂-Mangel im Zustande völliger oder teilweiser Bewusstlosigkeit während längerer Zeit ertragen werden kann. Warum es bei reinem O₂-Mangel in der Einatemungsluft erfahrungsgemäss doch rascher zu

Todesfällen kommt als bei CO-Vergiftung, welche gelegentlich über Stunden eine reversible Bewusstlosigkeit mit völliger Erholung aufweisen kann beruht nach eigenen Erfahrungen z. T. darauf, dass der CO-Vergiftete in der Regel Zeit hat, sich schützend hinzulegen, während der akute O_2 -Mangel wegen der durch die grössere Atmung sich aufstropfenden Alkalose ein plötzlicheres kampfärgeres Geschehen darstellt. So stürzen solche Patienten ohne vorherige Umschau bewusstlos hin und ersticken oft wegen atemungsbehindernder Körperlage wie im zitierten Ballonunglück, also Vorkommnisse, welche bei CO-Patienten wegen häufigen Fehlens des bewussten Zusammenfallens seltener sind. Im Gegensatz zur O_2 -Mangelatmung kommt es bei der CO-Vergiftung im Koma aber zu einem progressiv sich verkleinernden Atemvolumen mit praeternormaler stark ansteigender hypoxischer Sauerung des Blutes und der Gewebe. Durch diese Sauerung wird aber gleichzeitig die O_2 -Spannung (bei gleichbleibender prozentualer O_2 -Sättigung) erhöht. Die Acidose aber ermöglicht wiederum eine viel bessere Ausnützung des Blut- O_2 bis auf Werte von voraussichtlich 15 mm Spannung herab, allerdings bei ausgebliebenem Bewusstsein, während es also bei reiner O_2 -Mangelatmung im Gegenteil zu einer alkalotischen bedingten Erleichterung der O_2 -Ausnützungsmöglichkeit für das Gehirn gekommen ist, bei gleichzeitiger Linksverschiebung der O_2 -Bindungskurve was einer schädlichen Potenzierung gleichkommt.

Gleichzeitig aber kennt doch jeder Kliniker auch die schweren Schäden und die vielen Todesfälle, die sich bei CO-Vergifteten leider so häufig ergeben.

Umgekehrt aber können Menschen unter Umständen stundenlang in hypoxischem Zustande komatös verweilen (gleich wie CO-Vergiftete) ein normales oder gar saures Blut-pH-wert ge-
-B bei erscheinungsschweren Zuständen Haldane macht auf S. 204 darauf aufmerksam, und ich selbst verweise nochmals auf die 50jährige Asthmikerin, welche postoperativ über 1 Stunde lang tiefst asthmatisch-cyanotisch und bewusstlos dalag ohne plötzlichen Schaden zu nehmen.

Darum bedarf es also nicht der Haldane'schen Annahme einer normalen O_2 -Spannung (mit reduzierter O_2 -Menge) für die Erklärung der relativ langezeit erhaltenen Atemfähigkeit bei der CO-Vergiftung. Die Atmung kann vielmehr selbst bei

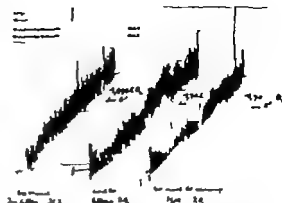


Abb. 22. Linke Kurve zeigt den O_2 -Verbrauch und die Atemkurven (Knipping-Apparat) über die Zeit. Mittlere Kurve: Atemkurve unmittelbar nach 30' CO-Atmung (5%). Rechte Kurve: Atemkurve 40' nach Beendigung der CO-Atmung. Die initiale Hypoxie und die Verkleinerung des Atemvolumens sind Ausdruck der gelagerten O_2 -Schuld (Abnahme der hypoxisch angeblähten Mischalve).

sehr niedrigen O_2 -Spannungen lange erhalten bleiben, sofern nur wie bei der CO-Vergiftung der Säuregrad des Blutes erhalten bleibt oder sogar zunimmt, ganz im Gegensatz zur Höhenatmung. Gleich wie unser kurzer Hyperventilationstest bei milder CO-Vergiftung zum sofortigen alkalotischen Koma führt, so bedingt auch eine in stark O_2 -armer Luft ausgeführte Ruhehyperventilation rasches Koma. Der Unterschied besteht also im Wesentlichen in der stark alkalotischen pH-Verschiebung bei der Höhenatmung im Gegensatz zum sauren Blute des sich selbst überlassenen CO-Patienten.

Die Gas-Stoffwechsel bei mittelstarker CO-Vergiftung

Aus unseren wenigen Eigenversuchen scheint der O_2 -Verbrauch in Zuständen von 30-50%iger CO-Vergiftung nur unwesentlich verringert (S. Abb. 22). Wohl aber ist uns aufgefallen, dass die Atemkurven in einem O_2 -am Knipping-Apparat gemessen, ein deutlich kleineres Totalvolumen ergeben. Diese Abnahme des Reatemolumens nach abgeschlossener CO-Atmung verdeutlicht sich sogar mit Latenz.

In Abb. 22 ist ein Beispiel einer solchen Atemregistrierung im Liegen sofort und 40 Minuten

Pulsfrequenz

80

70

60

■ 10 20 30 40 50 Zeitdauer der
CO-Atmung / Min

Abb 23 Regelmässige Anstiege der Ruhepulse mit zunehmender CO-Atmungszeit in Liegelage. Durchschnitt von 9 Versuchspersonen

nach Beendigung der halbstündigen Atmung von 50% CO in Luft gegeben. Aus unseren Versuchen ergibt sich das unerwartete Ergebnis, dass der O₂-Verbrauch trotz des um nahezu 1/3 kleiner gewordenen Totaleatemvolumens praktisch gleich gross geblieben ist. Die Versuchspersonen mussten vor dem Versuch 3 Stunden lang vorliegen um ihr Ruheatemvolumen als Ausgangswert vor dem CO-Versuch zu hefern.

Der Grundsatz bei 30%iger CO-Vergiftung:

geprüft wurden männliche Versuchspersonen

	vor Versuch	nach CO-Atmung
a) 30 j	+3%	+20%
b) 47 j	+7%	+12%

Versuchsperson a) atmete 33 ein 5% CO-Luftgemisch. Versuchsperson b) atmete 48' ein 5% CO-Luftgemisch.

Die Versuche wurden jeweils im direkten Anschluss an die CO-Atmung am kleinen Knippungs-Apparat ausgeführt.

Bei ruhig liegenden Menschen scheint sich demnach schon nach leichteren CO-Vergiftungen von nur 30% Blut-CO-Gehalt mit der Zeit eine O₂-Schuld einzustellen, die dann durch eine reine O₂-Atmung rasch getilgt wird. So kommt es zu einer erheblichen Verkleinerung des Atemminutenvolumens wie dies aus der Abb 22 hervorgeht, wo das Atemvolumen um 30% abnahm.

Damit ist wieder ein Hinweis dafür gegeben, dass das erste Organ, welches unter der CO-Einwirkung in Mitleidenschaft gezogen wird, das Gehirn sein muss und nicht primär andere innere Organe, ansonst der O₂-Verbrauch schon in den leichteren Stadien der CO-Vergiftung zu sinken begänne.

Zu unseren CO-Versuchen bis zur 30%igen Hb-Sättigung ist noch zu sagen, dass ausser einer ge-

wissen Dösigkeit leichtem Kopfschmerz im Liegen (mit der beschriebenen Kopfströmung und dem höheren Ruhepuls) beim gewöhnlichen Arbeiten eine massig starke Dyspnoe auftrat. Diese machte sich bei mir besonders geltend, als ich ersuchsweise mit dem Fahrrad kurz nach einem Eigenversuch nach Hause fuhr. Die kleine Anhöhe, die dabei zu erstiegen war, musste ich diesmal wegen Atemnot erstmals zu Fuss erklimmen. Dafür hatte ich nachher die Genugtuung, dass die Erholungszeit (gegenüber früheren und auch späteren CO-Versuchen ohne anschliessende stärkere körperliche Betätigung) verkürzt wurde.

Warum ist das Ruheatemvolumen bei CO-Atmung anfangs leicht vergrössert?

Unsere Versuchspersonen, bei welchen wir das Atemvolumen während der Atmung eines 5%igen CO-Luftgemisches massen, zeigten in den ersten 20 Minuten einen leichten Anstieg des Atemminutenvolumens, der gegen das Ende der gewöhnlich 30-35 Minuten dauernden CO-Atmung wiederum einer Verkleinerung bis unter die Werte der Norm Platz machte. Ein Beispiel ist in Abb 4

Nimmt man die Erklärung Haldanes an, wonach die O₂-Spannung im CO-Blute eine normale sei, weshalb selbst bewusste CO-Patienten kein vergrössertes Atemvolumen aufweisen, so ist diese ungefähr 10%ige Steigerung des Ruheatemvolumens unserer ausgeruhten Versuchspersonen während der ersten Hälfte der CO-Atmung nicht erklärlich. Es sieht vielmehr so aus, also ob die verminderte O₂-Spannung im ZNS im Wachzustande sich auszuwirken beginnt, sodass eine Stressreaktion mit Aussendung zentraler Impulse ausgelöst wird. Hierfür sprechen einmal das subjektive Hungergefühl mit dem gelegentlich geröteten Gesicht, das leichte Zittern der Finger und die leichte Pulsfrequenzerhöhung in Abb 23 und die schon bald mit zunehmender CO-Atmungszeit auftretenden Pulsfrequenzerhöhungen dargestellt als Durchschnittswerte von 9 gesunden liegenden Versuchspersonen. Sie begannen schon in den ersten 10 Minuten der CO-Atmung.

Zorn hatte 1972 an Ratten, Kaninchen und Katzen den interessanten Nachweis erbracht, dass die Abnahme des O₂-Partielldruckes im Gehirngewebe bei CO-exponierten Tieren nahezu reziprok linear dem Anstieg des CO-Sättigung in Blut verläuft. Diese Senkung des O₂-Partielldruckes

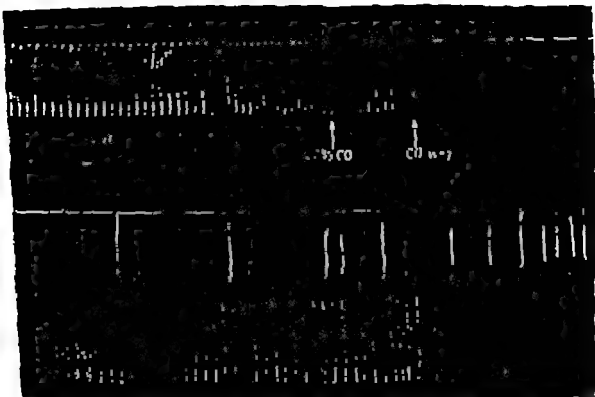


Abb. 24 zeigt den nach CO-Atmung bald eintretenden Atemstopp in Expirationsstellung bei markotierten Kanarienvögeln und Meerschweinchen mit langer Apnoe.

dann nur vereinzelt, immer rascher auftretenden Schenkelstößen (untere Reihe), bei sich allmählich wieder normalisierender Atmung (obere Reihe).

im Gewebe erfolgt schon durch niedrige CO-Konzentrationen des Blutes.

Für eine solche Auffassung sprechen indirekt auch die Versuche meines Schölers Lehmann an markotierten Meerschweinchen und Kanarienvögeln bei welchen nur der Einfluss der Narkose diese Stressreaktion trotz Atmung eines 20%igen CO-Luftgemisches ausbleibt. Es kommt vielmehr bei diesen tracheotomierten Tieren einfach zu einem plötzlichen Stillwerden der Atmung (s. Abb. 4).

Gleich erliefen seine Versuche wenn solchen markotierten und tracheotomierten Tieren 1 Natriumnitrit injiziert wurde wiederum setzte die Atmung ohne ortsbegrenzende Vergrößerung plötzlich aus (s. Abb. 25). Das Natriumnitrit hatte wie postmortale Blutuntersuchungen zeigten, die Bildung von Methämoglobin bedingt das für den O₂-Transport völlig wegschließt. Es konnte gleich wie das Blutplasma in noch rein physikalisch geringsten Mengen O₂ aber unter normalem Druck, auf

nehmen und deshalb die intraarteriellen Chemorezeptoren nicht im Sinne eines O₂-Mangels erkennen. Dagegen erhielt nun das Atemzentrum ungenügende O₂ Mengen und versagte ohne Auslösung von Warnreaktionen.

Comroe Schmidt fanden schon 1938 dass ein 60%ig CO-belegtes aber dann kurz mit O₂ durchperlit Blut den tierischen Carotids-Sinu nicht mehr entgegnete zum Aussenden atemsteigernder Impulse ans Atemzentrum, im Gegensatz zu einem



Abb. 25 zeigt den plötzlichen Atemstopp in Expirationsstellung aus normaler Atmung heraus bei 1. Natriumnitrit Injektion beim Kanarienvogel (markotiert).

Tabelle 3

Durchschnittswerte Atemvolumina Liter/Minut

Versuchspersonen	In Luft (l)	Luft+CO ₂	Nach CO-Atmung Luft+CO ₂
1 47 j. ♂	4,8	mit 3% CO ₂ 9,5	mit 3% CO ₂ 9,5
2 33 j. ♂	5,8	mit 5% CO ₂ 20,0	mit 5% CO ₂ 17,0
3 27 j. ♂	4,6	mit 6% CO ₂ 15,4	mit 6% CO ₂ 17,0
4 27 j. ♂	5,3	mit 7% CO ₂ 17,8	mit 7% CO ₂ 17,0
5 36 j. ♂	5,0	mit 7% CO ₂ 17,5	mit 7% CO ₂ 32,0
Durchschnittswerte	5,0	16,0 l	18,5 l

CO-durchspülten Blute weil im letzteren kein plasmatischer O₂ mehr war und die Erregung des Carotis-Sinus wiederum auftrat.

Diese Verhältnisse eines kaum oder nicht vergrößerten Atemvolumens bei Einnahme geringer CO-Mengen gelten nur für den ruhenden Körper oder den narkotisierten. Ein sich bewegender Mensch verspürt nach CO-Einnahme verstärkte Atemnot und weist damit eine Dyspnoe Atmung auf, da die Muskelsäure für Erregung des Atemzentrums sorgt. Sie verschwindet bei der CO-Vergiftung sowie auch in der Höhe unter Einfluss des O₂-Mangels langsamer als üblich weshalb das Atemvolumen durch eine gleiche Anstrengung sowohl während der CO-Vergiftung als auch in der Höhe viel stärker zunimmt und einer längeren Erholungszeit bedarf als im Normalfall.

Zu verminderten Ansprechbarkeit des Atemzentrums gegenüber CO bei CO-Vergiftung

Eigene Versuche mit 5 verschiedenen liegenden Versuchspersonen zeigten, dass bei nur 30%igen Blut-CO-Befall die Ansprechbarkeit des Atemzentrums gegenüber dem CO₂-Reiz noch nicht abgenommen hat, fast alle zeigten bei Einnahme eines 3-7%igen CO₂-Luftgemisches eine prompt auftretende und prozentual ca. gleichbleibende Steigerung des Atemvolumens wie dies vor dem CO-Versuch der Fall war. Es war also das Atemzentrum bei diesen leichten Graden der CO-Vergiftung noch durchaus in der Lage auf CO₂-Reiz normal zu reagieren (s. Tabelle 3).

Auch das Blut-pH war in diesem Stadium noch unverändert (5 Versuchspersonen). Dagegen konnte gelegentlich sogar ein stärkerer atmungssteigernder CO₂-Effekt im Stadium dieser CO-Vergiftung festgestellt werden.

In Tabelle 2 sind 5 Beispiele zusammengestellt, worunter in 2 Fällen ein stärkerer atmungssteigernder CO₂-Effekt im CO-haltigen Organismus vorliegt. Da O₂-Spannung und Menge im CO-Haemoglobin herabgesetzt sind, muss sich nach meiner seit 1942 vertretenen Ansicht die CO₂ in leichteren noch nicht längere Zeit bestehendem CO-Blut eher stärker auswirken bei hohem O₂-Druck dagegen geringer.

Im Gegensatz zu diesen leichteren CO-Vergiftungen bewirkt aber ein starker CO-Befall im späteren Stadium mit Koma eine geringe Ansprechbarkeit des Atemzentrums gegenüber dem CO₂-Reiz. Deshalb wird dann das Atemvolumen kleiner unter starkem Anstieg der Blut- und Gewebssäuerung. Diese Tatsache ist bekannt und findet sich u. a. auch bei Zorn. Die Schuld an dieser Acidose trägt der O₂-Mangel unter welchem das Atemzentrum zu versagen beginnt. Erst stärkste Säurereize vermögen in diesem Stadium noch vereinzelte grosse „Seufzer“ Atemzüge hervor zu bringen. Werden Meeresschweinchen welche in CO-Luft bewusstlos hinfallen und keinerlei Atembewegungen mehr zeigen, in diesem Stadium in eine kleine O₂-Überdruckkammer eingeschleust, so erholt sich die Atemtätigkeit wiederum über den Weg einer Inhalation, grossen und frequenten Atmung. Selbst wenn durch akute CO-Vergiftung bei Zufuhr von 20% CO die Meeresschweinchen innerhalb einer Minute bewusstlos und völlig atemgelähmt wurden, konnten sie sich zu unserem Erstaunen spontan erholen, wenn sie sofort in reine Luft gebracht wurden. Alsdann sieht man, dass nach ungefähr 20-30 Sekunden ein erster tiefer Atemzug erfolgt, nach weiteren ca. 15 Sekunden ein zweiter. Dann folgen sich diese tiefen „Schnappatemzüge“ immer häufiger und nach einigen Minuten ist die Atmung einigermaßen nor-

mal und regelmäßig geworden. Die Tiere erholen sich spontan!

Abb 24 zeigt eine unserer vielen Atmungskurven von auf obige Weise CO-ergifteten Meeresschweichen mit spontaner Erholung.

Das Blut-pH von 8 solchen Tieren ging im Momente des längsten Atemstopps von 7,54 auf 7,22 herunter! So stark ist die Lähmung des Atemzentrums in diesem Stadium! Durch diese starke Säuerung kann aber der O_2 bis auf allergeringste Spuren im CO-Körper verbraucht werden, im Gegensatz zur Alkalose welche im Gegenteil die zentrale Verwertbarkeit des O_2 verschlechtert. Letzteres ist wohl der Grund für das unterschiedliche Verhalten von Patienten im O_2 -Mangel und bei CO-Vergiftung.

Unsere Meeresschweichenversuche stehen im Gegensatz zu Angaben von Haldane der fand, dass Mäuse die in einem Überdruck von 2 Atm. O_2 und 1 Atm. CO sich normal verhalten beim Ausschleusen jedoch akut an CO-Vergiftung starben. Untersuchungen meines Schülers Tromp konnten dies zwar für Mäuse all bestätigen, nicht dagegen für die größeren Meeresschweichen welche sich spontan nach schwerster CO-Vergiftung (in Kurzversuchen) in Frischluft erholen konnten. Es muss dies mit dem höheren Grundumsatz kleiner Lebewesen zusammenhängen, wo sich ein O_2 -Mangel stärker auswirken muss. Diese spontane Erholungsfähigkeit nach akuter CO-Vergiftung bis zum Koma wurde 1969 nun auch durch Schüttek an Ratten bestätigt.

Die Atmanhaltmöglichkeit während der CO-Vergiftung

Interessanter bestimmten wir bei unseren CO-Versuchen das maximale Atmanhaltevermögen in der Vorstellung dass dieses erheblich herabgesetzt würde. Wir waren anfänglich recht erstaunt dass dies nicht der Fall war. So betrug beispielsweise mein maximales Atmanhaltevermögen in dem Versuch 85 in Liege Lage nach 2 Minuten der Atmung eines 7%igen () CO-Luftgemisches immer noch 83 () Durchschnittswerte aus je 3 Versuchen

Die Erklärung für diese zunächst für uns unerwartete Tatsache wird folgende sein: wohl sind die Erythrocyten nur je zu Hälfte mit O_2 gefüllt (die oberste Hälfte jeweils durch CO) sie werden aber nach Teilentleerung in den Kapillaren durch die Spannung des O_2 der Lungenluft rasch

immer wieder bis zum möglichen Ende der O_2 -Bindungskurve nachgefüllt. Dies aber geschieht im Gegensatz zur Norm viel leichter weil weniger O_2 -Spannung hierzu nötig ist. Da aber gleiche O_2 -Spannung und Menge in der Lungenluft vorliegen wie in der Norm, erhält sich auch die ursprüngliche Atmanhaltefähigkeit beim mittelstark CO-vergifteten Menschen.

Zur Genese des CO-Kopfschmerzes

Allen unseren 12 Mitarbeitern ist immer wieder die sehr bekannte Tatsache des lastigen Kopfschmerzes im unmittelbaren Anschluss an die CO-Vergiftung aufgefallen der oft über einige Stunden anhält. Obwohl er sich subjektiv gleich äussert wie der Höhenkopfschmerz, scheint mir doch diese Genese eine andere denn während der Höhenkopfschmerz nach eigenen Untersuchungen (1964) ein allotischer Kopfschmerz ist der durch Entweichen von 4-5% CO_2 innerhalb von 20 Sekunden vorübergehend ganz behoben werden kann ist dies beim CO-Kopfschmerz durchaus nicht der Fall. Man darf wohl für letzteren mit Wolff das konsequente Ödem der Schädel (und Gehirns) Arterien-Adventitia-Schichten für diese lang anhaltenden Kopfschmerzen verantwortlich machen welches auf Grund der starken arteriellen Kopfgefässerweiterungen entsteht und welche letztere auch schuld sind am subjektiven Hitzegefühl und der sichtbaren Gesichtsrötung. Die Genese dieses Kopfschmerzes ist also wesentlich mit den durch 1 Histamininjektion ausgelösten Kopfschmerzen, sie werden ebenfalls (nach eigenen Untersuchungen) durch CO_2 -Atmung nicht beeinflusst.

Diese deutliche Kopfrötung mit dem bald einsetzenden Kopfschmerz war den früheren Hausfrauen anlässlich ihrer Bügelarbeiten mit den holz-kohlegeheizten Bügeleisen allgemein bekannt und beruhte auf leichter CO-Vergiftung. Es ist dieser Kopfschmerz übrigens das erste subjektive unangenehme CO-Frühsymptom. Nach eigenen Untersuchungen wird dieser CO-Kopfschmerz (wie übrigens auch der Histaminkopfschmerz) durch O_2 -Atmung recht lange Zeit nicht beeinflusst (dies steht im Gegensatz zur Angabe von Zorn der ein sehr rasches Nachlassen des Kopfschmerzes durch O_2 -Atmung angibt). Daneben bemerkten wir in unseren CO-Versuchen als weiteres Frühsymptom zusammen mit der Kopfrötung

ein leichtes Zittern der Finger und einen leichten Pulsanstieg

CO-Wirkung in der Höhe

Die öfters vertretene Meinung eine leichte CO-Vergiftung müsste sich in der Höhe viel verdrücklicher auswirken als in der Talluft (s. z.B. Jordi, aber auch bei Haldane auf S. 239) hat sich uns zunächst nicht bestätigt. Wir haben 6 Mitglieder einer eigenen Jungfrauochsexpedition im Tal (560 Meter über Meer) und am ersten Tage auf Jungfrauoch (3450 Meter) CO in ausgeruhter Liege lagen sitzen lassen um Tal 5%, auf Jungfrauoch 4,5% (beide Werte unreduziert) um durch ev. Blockierung der Carbanhydrase den sonst häufigen, alkalotischen Kopfschmerz zu vermeiden oder zu unterbrechen. Zwar ist uns letzteres nicht gelungen dagegen aber hat keiner der Teilnehmer irgendeine Änderung in seinem Befinden während oder nach der 15minütigen CO-Atmung empfunden, wahrscheinlich weil keine Arbeitsleistung erbracht und Liegelage innegehalten wurde. Es ist dies nach unserer neuen Vorstellung über die Wirkungsweise der CO-Vergiftung verständlich da das CO ja nur die oberste durch O₂ in der Höhe oben nicht oder nur mit Mühe belegte Partie der Hb-O₂-Sättigungskurve besetzt. Dies aber bedeutet für den Höhenorganismus keinen wesentlichen Abbruch.

Douglas u. Haldane atmeten eine O₂-arme Luft gemisch entsprechend einer Höhe von 5000 m. In diesem Zustande konnte eine Blut-CO-Sättigung von 25% ohne merkliche Störung ertragen werden (auf S. 279 des Buches von Haldane). Diese Angabe steht im Gegensatz zu dem Postulat auf S. 239 von Haldane, welches oben erwähnt wurde.

Dagegen wurden wiederum, wie am Tal unsere CO-Versuchspersonen nach ungefähr 6-8 tiefen und raschen Hyperventilationsatemzügen vorübergehend bewusstlos wie dies früher beschrieben und erklärt wurde.

In ähnlicher Weise hatten Haldane und Smith (1897) zu ihrem grössten Erstaunen gefunden dass CO-teilvergiftete Mäuse O₂-Mangel nicht schlechter ertrugen als normale kontrollierte. Dies gab ja den Anlass zur systematischen Untersuchung über die O₂-Bindungskurven eines teilweise durch CO-belegten Blutes.

Wohl aber müsste es dann in grösseren Höhen zum Unglück kommen wenn es durch stärkeren CO-Einbruch ins Blut nicht nur zur Belegung der

O₂-freien oberen Hb-Partien sondern noch zu einer zusätzlichen massiveren Verdrängung des O₂ aus der verbleibenden Hälfte der Bindungskurve des Hb käme. Hier müsste die höhenbedingte Steigerung des Atemvolumens zur besprochenen alkalotischen Rechtsverschiebung (=Hemmung) der O₂-Verwertungskurve im Gehirn und damit zur rascheren Bewusstlosigkeit führen.

Für eine solche Auffassung sprechen übrigens unsere besprochenen Selbstversuche auf Jungfrauoch (3450 m). Daneben konnten schon bei einer Blut-CO-Sättigung von nur ca. 15% die gleichen Komasympptome durch 6-8 tiefste Hyperventilationsatemzüge ausgelöst werden, weil in der Höhe schon eine leichte Alkalose vorliegt. Im Tale bedurfte es einer 25%igen CO-Sättigung für die gleichen Hyperventilationssymptome.

In ähnlicher Weise wurde ebenfalls unsere 40-jährige weibliche Versuchsperson im Zustande einer 30%igen CO-Blutsättigung im Tale nach 4 1/2 minütiger Atmung eines 10%igen O₂-Gemisches (mit N₂) bewusstlos weil durch die Steigerung des Atemvolumens von 7 lt pro Minute auf 11 lt es zu der verderblichen Alkalose gekommen war welche sich im CO-Blut so drastisch auswirkt. So wurde die Schwelle des Bewusstseins unterschritten. In der nachfolgenden Luftatmung geschah baldige spontane Erholung im Liegen. Im vorhergehenden Kontrollversuch mit Atmung eines 10%igen O₂-Gemisches (vor der CO-Vergiftung) trat keine Bewusstlosigkeit auf wohl aber die erhebliche Steigerung des Atemvolumens. Dadurch aber konnte die Blut-O₂-Sättigung deutlich zunehmen im grossen Gegensatz zum CO-Versuch. Bei letzterem brachte die Hypernimmung nicht nur keinen Gewinn, sondern im Gegenteil nur einen alkalotisch bedingten O₂-Verlust für das Gehirn.

Werden durch die menschlich CO-Vergiftung auch Gewebs Katalysatoren gekennnt?

Diese in der Literatur gelegentlich vertretene Ansicht (z.B. Meldrum u. Roughton (1934) ferner Warburg (1926) und andere) stützt sich allerdings nur auf ausserordentlich starke CO-Konzentration bei Gewebsexplantaten oder Hefezellen mit Gasgemischen die weit über 50% CO aufweisen was bei der menschlichen CO-Vergiftung praktisch nie vorkommt (vergleiche hierzu die bereits beschriebenen eigenen Versuche meines Schülers Schlüpfer).

Warburg hatte 1926 zudem nachgewiesen, dass die Affinität des CO für Cytochromoxydase niedrig ist und nur ca 1/10 derjenigen an O_2 entspricht.

Ein starkes Argument gegen d. Ansicht von menschlicher G. cbs-Katalysoren-Vergiftung durch CO erblicke ich in der Arbeit von Martland u. Martland = 2 Fälle tödlicher CO-Vergiftung schwangerer Frauen seziert u. den ohne dass im fötalen Blute CO-Spuren nachgewiesen werden konnten

Offensichtlich genügt selbst diese hohe Blut-CO-Sättigung noch nicht, um die Placentarschranke welche für die fötale O_2 -Versorgung gut passierbar ist zu überschreiten.

Es ist dies erstündlich wenn man sich an die ungeheuer geringen CO-Drucke erinnert, die zur 60-70%igen (gewöhnlich tödlichen) Sättigung des Blutes führen. Sie liegen unter einem Millimeter Hg.

Die gleiche Ansicht, dass das CO unter so niedrigen Drucken an Haemoglobin gebunden sei, da es bei der CO-Vergiftung des Menschen praktisch nicht ins Gewebe hinein diffundieren könne, wird auch durch Ellinger gestützt. Letzteres könnte erst dann eintreten, wenn der Partialdruck des CO in der Einatemungsluft sehr hoch wäre, was aber wegen fast augenblicklicher totaler Blockierung der Erythrocyten ebenso rasch zum sofortigen Tode führen könnte, wie die Na $\frac{1}{2}$ mit Injektion mit sofortiger Blockierung der Erythrocyten im Tierversuch. Auch im letzteren Falle werden in der kurzen Zeit kernernen Zellkerne gehemmt, weshalb es in der O_2 -Überdruckkammer nicht zur sonst raschen Schädigung solcher Tiere kommt.

Übrigens ist die CO-Affinität für Cytochromoxydase nur etwa 1/10 von jener für O_2 . Die Verhältnisse für Cytochromoxen noch unklar (nach Kreuzer).

Desgleichen sprechen Haldane Versuche mit Mäusen, welche sich in einer Überdruckkammer von 3 Atm in einem Gemisch von 1/3 CO und 2/3 O_2 durchaus normal verhielten und erst beim Ausschleusen einer bewussten Zusammenbrachen und bald starben, gegen die Ansicht einer Hemmung von Gewebekatalysatoren durch CO. Diese Versuche konnte mein Schüler Temp in einer Dissertation vollständig bestätigen.

Haldane hat ferner an Hb-freien Insekten (Käferschaben) die Ungefährlichkeit des CO selbst

in 10%iger Konzentration (1 mit 90% O_2 bewiesen, indem diese Tiere 14 Tage lang in dieser Atmosphäre ohne jegliche Störungen leben konnten (auf S. 37 seines Buches Respiration, 1935).

Eigene Versuche mit Meerschweinchen, welche in einer 10%igen CO-Luft in kurzer Zeit komaös und blig atemgelähmt hinfielen, verliefen ebenfalls äusserst interessant, sofort in einen O_2 -Überdruck von 3 Atm gebracht, erholten sich diese Tiere in kurzer Zeit und konnten nach ungefähr 10 Minuten wiederum ausgeschleust werden ohne sichtbaren Schaden aufzuweisen. Damit musste der Grossteil des Blut-CO im O_2 -Überdruck wiederum abgeraucht sein. Blutuntersuchungen ergaben nur noch CO-Mengen von 15-20%.

Primäre oder sekundäre Herzschädigung durch CO

Haldane (auf S. 237 seines Buches „Respiration“) schloss aus dem raschen Kollabieren CO-vergifteter Menschen bei Gehirnischen und seinem Eigenversuch mit der Vergiftung zum Hinfallen und dem Überlebensgefühl auf eine primäre CO-bedingte Herzschädigung, welche er für diese Zustände anschaufte. Dafür schien auch zu sprechen, dass die Atmung selbst in bewussten Zuständen noch lange erhalten bleiben konnte. Die bei überlebenden CO-Patienten häufig beobachteten Herzrhythmusstörungen schienen ihm eine Bestätigung für diese Ansicht.

W selbst und durch unsere zahlreichen Tier- und Eigenversuche wenigstens für CO-Kurzversuche zu anderer Ansicht gelangt.

Leitet man z.B. ob oben dichten mit Abzug erhaltenen Meerschweinchenkäfig ein 20%iges CO-Luftgemisch so werden die Tiere in kurzer Zeit bewusstlos. Hierbei hört die Atemtätigkeit sofort auf, bei noch längere Zeit schlagendem Herzen. Versetzt man nun diese bewussten, atemgelähmten Tiere in eine kleine und daher rasch auf Überdruck zu bringende Überdruckkammer mit 2-3 Atm einem O_2 , so erholen sich sämtliche dieser bewussten und atemgelähmten Tiere innerhalb weniger Minuten. Sie begannen zwar mit einer grossen und frequenten Atmung, welche sich zur Tilgung der O_2 -Schuld (mit der Anhäufung hypoxischer Stoffwechselsäuren) einstellt, doch schon bald normalisiert sich die Atmung und die Tiere erheben sich und gehen wiederum in normaler Weise in der Kammer

herum. Hier genügt also das passive Elupressen von O_2 in die Atemwege zur Wiederbelebung. Ein Beweis, dass hier unter CO eindeutig das Atemzentrum und das Bewusstseinszentrum primär gelitten hatten und vorerst nicht das Herz.

Auch Zorn berichtet, dass das Kreislaufversagen meist später einsetzt als das Versagen der Atmung.

Interessante Nebenbeobachtung: sogar schon 5 Minuten nach der Einschleusung konnten diese Tiere unbeschadet wiederum in gewöhnliche Luft ausgeschleust werden, ohne dass sich die zunächst zu erwartenden Störungen der Hypoxie wieder eingestellt hätten! Blutuntersuchungen dieser Tiere zeigten, dass auch bei ihnen nur noch unter schwellige CO-Mengen von höchstens 20% vorlagen. Damit ist der Beweis erbracht, dass durch den O_2 -Überdruck von 7 Atm das CO in kurzer Zeit zum Grossteil aus hypoxisch gesäuerte Blut wiederum verlassen hat.

Registriert man an einem narkotisierten Meer-schweinchen oder Kaninchen die Atemtätigkeit in Normalluft, so erlebt man wiederum bei Zufuhr von 5-10% CO in kurzer Zeit ein völliges Stillwerden der Atemtätigkeit in der CO-haltigen Luft, während das Herz weiter schlägt. Unterbricht man jetzt die CO-Atmung, so treten nach 15-30 se-
ndiger Pause vereinzelt „Seufzer“ Atem

in immer kleiner werdenden Intervallen bis nach wenigen Minuten die Tiere wiederum eine normale Atemtätigkeit aufweisen (s. Abb. 19).

Während Haldane (auf S. 74) seines Buches „Respiration“) die Ansicht vertritt, dass CO einen genügenden Grad der Anoxie bewirke zur Erzielung einer primären Herzheldigung, während das Atemzentrum noch antwortet, verließen unsere Tierkürzversuche also umgekehrt, was aus Abb. 24 deutlich hervorgeht. Offensichtlich ist das Herz mit seiner Arbeit bei O_2 -Mangel geschützt als das Gehirn.

Eine interessante Nebenbeobachtung schien zunächst jedoch für ein primäres Erlahmen des linken Herzens zu sprechen, akut durch CO bewusstlos hingefallene und ertrunkene Meer-schweinchen konnten nicht passiv durch Thorax-kompressionen bestrahlt werden, indem keine Luft die Trachea verließ, auch nicht bei vorangegangener Anlage einer Tracheotomie. In diesen immer wieder beobachteten Fällen trat zuerst als Ausdruck reiner Schädigung des Herzkam-

mer auf, wobei der rechte Ventrikel weiterhin Blut in die Lunge pumpt und es zu einer Art kardialen Athmas käme.

Inzwischen hat Pettinati in Hundeversuchen nachgewiesen, dass es bei 80%iger Blut-CO-Sättigung vor dem Herzkammerflimmern zum Ansteigen des Blutdruckes in der Lungenschlagader auf das 2-3-fache kommt. Hier hatte zweifellos der linke Ventrikel präterminal zu versagen begonnen, was begreiflich erscheint. Da unsere Beobachtung jedoch nach vorangegangener Aderläsen nicht mehr gemacht werden konnte, drängte sich noch eine andere Erklärung auf. Es muss sich hierbei auch um den Effekt des starken Muskel-tonusschwundes der Atemmuskulatur, akut CO-vergifteter Tiere handeln, weshalb der Thorax in stärkste Expirationsstellung zurückfällt. Dabei wirken die nun blutüberfüllten Lungen (deren Gefäßquerschnitte größer geworden sind) ganz gleich wie eine kardial gestaute Lunge. Beide können durch passive zusätzliche Thoraxkompression wegen Ventilmechanismus nicht beatmet werden, wohl aber durch tracheale Überdruckatmung.

An mir selbst habe ich nach Atmen eines 7,5%igen (!) CO-Luftgemisches in Liegelage während 5 Minuten eine Blut-CO-Sättigung von 55% erlebt. Während ich gut sprechen und auch schreiben konnte (letzteres nur etwas zitterig), kollabierte ich anal beim Versuch herumzugehen und verlor jeweils nach vorangegangen Übelkeitgefühl das Bewusstsein. Hierbei sank der Blutdruck jedesmal auf nicht mehr messbare Werte, erhöhte sich aber in Liegelage mit passiver Beinhochlage in kurzer Zeit immer wieder. Dieser Versuch scheint mir dafür zu sprechen, dass es nicht primär das Herz war, das hier versagte, sondern das Vasomotorienzentrum und ganz allgemein die Empfindlichkeit des ZNS gegenüber O_2 -Mangel, worauf früher schon hingewiesen worden ist.

Auf diese Weise kann es natürlich auf die Dauer schon zu einer Herzheldilation kommen, die aber dann, meiner Meinung nach, als sekundäres Phänomen aufzufassen ist, indem die Coronardilatation infolge des Blutdrucksturzes leidet und sekundär zum Schaden führen kann und EKG-Veränderungen bedingt.

Im übrigen ist wegen der Muskelmischsaure arbeitenden Herzens die Möglichkeit einer besseren O_2 -Ausnutzung bis auf erheblich tiefere O_2 -Spannungen hinab gewährleistet im Gegensatz zum ZNS, welches wenigstens in Kurzversuchen

eine sehr erhebliche Abhängigkeit von den Bohr'schen O_2 -Sättigungskurven und der alkalotisch bedingten Erschwerung der O_2 -Verwertung auf ist, was gezeigt worden ist.

Auch die Tatsache, dass das Myoglobin nach Mähten eine besonders hohe O_2 -Bindungsfähigkeit hat, spricht für eine gewisse Resistenzmöglichkeit des Myocards gegen O_2 -Mangel. So liege beispielsweise die Halbsättigung des Myoglobins mit O_2 bei 4 mmHg, während beim Hämoglobin eine solche erst bei 30 mmHg vorliegt.

Warum wird eine chronische Anämie von 50 % Hb gut ertragen im Gegensatz zur 50 %igen CO-Blockierung der Erythrocyte

Aus der Literatur ist zur Genüge bekannt, dass chronisch erfolgende Hb-Verluste (parasitär, Chlorose, Perniciosa usw.) bis auf 50 % hinab ohne weiteres ertragen werden und sogar eine nicht zu schwere körperliche Arbeit gestatten. Selbst Patienten mit nur mehr 30 % Hb sind noch zu leichter Arbeit fähig.

Ich konnte 2 eigene Patienten untersuchen, welche mit chronischen Anämien in meine Behandlung traten. Eine 45jährige Frau mit inneren Hämorrhoiden, welche bei jedem Stuhlgang blutete, bei der Patientin ein Hb von 12 % nachwies. In diesem Stadium war sie nicht mehr gehfähig, gab aber im Liegen und Sitzen normal Auskunft und zeigte klinisch keinerlei vergrößerte Robestimmung. Durch Bluttransfusionen und Operation gelang diese Patientin in kurzer Zeit.

Ein 70jähriger Magencarcinom-Patient kam mit einem Hb von nur 9 %. Dieser Wert wurde wegen Zweifels monatelang in meiner Gegenwart durch die größte Laborabteilung noch 2mal wiederholt und ergab in der Tat „mal 9 %“ das 3. Mal 10 %. Gemessen mit dem Zeiss'schen Hämoglobinomometer. Auch dieser Patient konnte im Liegen eine durchaus normale Anamnese geben und zeigte wiederum keine Atemnot oder Dyspnoe Atmung im Liegen. Als er sich jedoch auf den Abort begeben wollte kollabierte er, erholte sich aber sofort wieder im Liegen. Nach Transfusionen konnte ich noch diesen Patienten erfolgreich operieren.

Im grossen Gegensatz zur chronischen Anämie ist also, wie dies Haldane und auch der beschriebene Eigenversuch gezeigt haben, ein zu schneller Verlust von O_2 „vergifteter“ an der alleräußersten Schwelle seiner körperlichen Leistung. Er erhält sich so wie unsere beiden beschriebenen Patienten mit Hb-Senkung auf ca. 10 % hinab, ein Gehen ist nicht mehr möglich wegen des baldigen Kollabierens.

Nach der Haldane'schen Erklärung ist für mich

das Problem nicht gelöst, weil Haldane beim CO-Patienten zwar eine normale O_2 -Spannung aber eine (wegen Teilbelegung durch CO) verminderte O_2 -Menge annimmt, weshalb ein solches CO-Blut seinen O_2 nur in kleinen Mengen, jedoch normal rasch am Gewebe abgeben könne.

Da nun aber beim CO-Patienten die Zahl der Erythrocyten im Gegensatz zum anaemischen Patienten nicht vermindert ist, sollte ein 40 %iger CO-Patient doch ungefähr gleich gut dastehen wie ein Anaemischer mit 40 % Hb.

Der Einwand, es hätte eben der chronisch-anämische Zeit zur Adaptation gehaut ist aber deshalb nicht stichhaltig, weil im Tierversuch eine Anämie im Akutversuch durch jeweiligen Plasmenersatz bis hinunter zu 10 % Hb ohne weiteres ertragen wird.

Nach der neuen Erklärung ist die Lösung dieses Problems einfach geworden. Im zu 50 % belegten CO-Blut sind nach Abb. 13 sowohl die biologische O_2 -Spannung als auch die O_2 -Menge ganz ausserordentlich stark herabgesetzt. Beides muss sich als Schaden potenzieren, denn O_2 -Druck mal O_2 -Menge entsprechen der biologischen Wirkung. Auch ist nach dieser Auffassung das so eindrucksvolle Bild raschster Bewusstlosigkeit durch kurzes Hyperventilieren bei CO-haltigem Blute besser erklärt.

Dabei ist der rein physikalische O_2 -Druck des CO-Blutes zwar normal, doch jedoch genügt die sehr kleine O_2 -Menge in keiner Weise für eine nur minimale Erhaltung der Funktion des Atemzentrums wie durch das sofortige Aufhören der Atmung durch Infusion von Na-Nitrit im Tierversuch gezeigt worden ist.

Umgebungs-Temperatur und Erholungs-rit bei CO-Vergiftung

In seinem Buche „Respiration“ machte Haldane auf S. 437 auf die Beobachtung von Kohlebergwerkarbeitern aufmerksam, wonach sich der Zustand von schwer CO-ergifteten Menschen verschlechterte, wenn sie an kalte frische Luft gebracht wurden, im Gegensatz zu windgeschützten wärmeren Räumen. Eine Erklärung hierfür gibt Haldane nicht.

In einer Insang. Dissertation liess ich meinen Schüler Hunziker Untersuchungen an je 54 Tieren mit CO-vergifteten Meerschweinchen anstellen, wobei 3 Gruppen bei Erholungslufttemperaturen von 4, 20° und 37° untersucht wurden. Da

ergab sich in Bezug auf das erste Herumgehen dieser primär durch 20% CO-Luftgemisch komatös gewordenen Tiere, dass die kürzeste Erholungszeit in 20° Luft auftrat (19.45 als Durchschnittswert), die weitaus längste in 4° Luft (49.15) und eine mittlere in 37° Luft (28).

Als Erklärungsversuch hierfür sei folgende Annahme gemacht. Die komatösen CO-Tiere verhalten sich einer küsseren Abkühlung gegenüber wie hibernierte Warmblüter. Sie erleiden keine periphere Vasoconstriction und kühlen deshalb wie der narkotisierte Organismus ab. Dadurch aber wird der O_2 viel leichter an das Hb gebunden und folgedessen erschwert an das Gewebe abgegeben. Umgekehrt dürfte man sich vorstellen, dass durch Erwärmung der komatösen CO-Tiere deren O_2 -Verbrauch gesteigert wird, wogegen die CO-Teilbelegung dieses Blutes eine zusätzliche O_2 -Meherversorgung nicht zulässt, denn durch die benötigte Hyperpnoe geschieht die anfangs beschriebene alkalotische Erschwerung der cerebralen O_2 -Verwertbarkeit.

Zur Frage der Vielgestaltigkeit der Symptome nach CO-Vergiftung

Der Satz von Ellinger (1931), wonach sich sämtlichen Autoren darüber einig seien, dass der Umstand der Nachkrankheitssymptome in keinerlei Beziehung stehe zur Schwere der akuten Vergiftung, wird allerdings durch Teleky bezweifelt. Er fand, dass der Prozentsatz der Nachkrankheiten bei den Fällen, die mit langdauernder Bewusstlosigkeit begannen, viel grösser ist als bei den anderen.

Unter der bisher entwickelten Ansicht, dass es bei der menschlichen CO-Vergiftung keine spezifisch toxische CO-Wirkung gibt, kann die so grosse Mannigfaltigkeit der beobachteten Spätsymptome ausserordentlich vereinfacht werden bei Berücksichtigung der Tatsache, dass das ZNS das primär leidendste Organ ist. Warum jedoch in einem Fall das Zuckerstoffwechselzentrum in einem anderen das Vasomotorenzentrum in einem dritten das Temperaturregulationszentrum in wieder anderen mehr geistige Funktionen betroffen sind, beruht meiner Ansicht nach auf der durchaus nicht immer einheitlichen Blutversorgung aller ZNS-Partien. Dies wissen die Neurochirurgen, seitdem man die cerebrale Angiographie macht, und dies weiss besonders auch der Pathologe, der die erschledenen Gefäss-Anomalien und -Pathologien sieht.

Nach Kreuzer treten CO-Schädigungen vor allem in Hirngebieten mit relativ schlechter Kapillarkirkulation auf, z.B. Globus pallidus, rote Zone der Substantia nigra und an Oberflächen der Hemisphäre.

So mussten wir selbst einen 41-jährigen Patienten begutachten, welcher nach mehrfachem Kollabieren anlässlich von Gasleitungsreparaturen schlussendlich eine tiefe Bewusstlosigkeit davontrug. Dies führte zu einer über Monate anhaltenden Störung seines Vasomotorenzentrums als alleiniges Überbleibsel der CO-Vergiftung. Dieser Patient wies immer bald nach dem Aufstehen starken und progressiven Blutdrucksturz bei normaler Herzaktivität auf und wurde von der Versicherung schlussendlich angenommen. Durch langes und systematisches Trainieren konnte das Vasomotorenzentrum wiederum erzogen werden.

Ich möchte also diese Vielgestaltigkeit der nervösen Ausfälle auf die hypoxische Noxe des ZNS zurückführen. Damit aber vereinfacht sich wiederum die Ansicht über die schädliche CO-Wirkung beim Menschen.

Zur Diagnose leichter CO-Vergiftung

Durch unseren Hyperventilationstest im Liegen tritt schon bei 25%iger CO-Vergiftung, welche noch recht wenig Allgemeinsymptome macht, nach wenigen Sekunden ein kurzdauerndes Koma auf, welches in wenigen Sekunden spontan beboben ist und nach unseren zahlreichen Eigenversuchen wegen der Kürze seiner Dauer als durchaus harmlos bezeichnet werden darf. Dieser einfache Test soll die Blutanalyse nicht erdrängen, sondern im wesentlichen zur Bereicherung der Frühdiagnostik beitragen.

Zur Frage der chronischen CO-Vergiftung

In der Literatur besteht verschiedentlich die Ansicht, dass es eine chronische CO-Vergiftung gäbe als Folge einer langen Kette leichter „Angiftungen“, so besonders durch Symansky (1936), Drinker (1938), Zorn (1965) u. a.

Der Begriff der chronischen CO-Vergiftung sollte nur auf solche Fälle bezogen werden und nicht auf chronisch gewordene Schädigung durch einmalige, starke CO-Vergiftung.

Mehrheitlich scheint nach dem internationalen Symposium der CO-Forschung von 1965 in Stuttgart die Meinung zu überwiegen, wonach es eine echte chronische CO-Vergiftung durch unter schwellige CO-Belegung des Blutes nicht gebe. Ny-

stören (zit. bei Schittnek) konnte bei Ratten, die er über mehrere Monate auf einem CO-Hb-Spiegel von 10-20% gehalten hatte, keinerlei histopathologische Veränderungen feststellen, desgleichen auch Ahlmark an Mäusen.

Die Vorstellung einer Vergiftung von Gewebskatalysen durch CO ist nach den früher angegebenen Argumenten zu verlassen, da die hierzu nötige CO-Spannung in solchen Fällen nie ausreichen würde.

Eine nur mittelstarke, immer wieder erfolgende CO-Vergiftung wird sich nach all dem Gesagten einiger während der Arbeitsleistung auswirken, so praktisch nur die verstärkte Arbeitsdyspnoe (Muskeilmilchsäure!) auffällt, als vielmehr in der darauf folgenden Rubepause. Diese führt zur Reduktion der während der Arbeit sich im Blut angelagerten Muskeilmilchsäure. Es kann dabei eine vorübergehende leichte Alkalose des CO-verarmten Blutes auftreten mit der beschriebenen schädlichen Wirkung im CO-Organismus. (Vergleiche einigungs geschilderten Hyperventilations-obsessiven bei leichtem Blut-CO-Befall.)

Je länger aber eine körperliche Anstrengung im Stadium *no 4* leichter CO-Vergiftung III frischer Luft anhält, desto rascher wird das im Blut verankerte CO durch die Muskeilmilchsäure und die grosse Atmung wiederum ausgetrieben. Folglich sind Zustände nach nur kurzer Arbeitsleistung bei mässig stark CO-vergifteten Patienten unter Umständen gefährlicher als nach länger anhaltender Arbeit (sofern die Personen sich wieder in Frischluft bewegen).

Wie bei körperlicher Anstrengung führt auch die Zufuhr von CO₂ zu einem ruhenden Patienten nur mittelstarker CO-Vergiftung zu einer grossen Atmung mit baldiger subjektiver und objektiver Besserung der Symptome wie wir das im Selbstversuch beschrieben haben. Daraus ergibt sich die bekannte Nützlichkeit der therapeutischen CO₂-O₂ Beatmung von CO-Patienten auf welche Haldane und auch Halderson hingewiesen haben. Im grossen Gegensatz hierzu wäre jedoch eine passive forcierte Hyperventilation nach dem beschriebenen äusserst gefährlich.

Gibt es eine Gewöhnung an leichtere CO-Vergiftung?

Haldane hat durch seine während längerer Zeit täglich vorgenommenen Eigenversuche diese Frage bejaht, indem mit zunehmender Gewöhnungszeit

die Symptome einer CO-Vergiftung später oder milder auftraten. Nach längerer Latenzperiode traten die CO-Symptome wiederum bei geringerer CO-Konzentration auf.

Im Eigenversuch konnte ich diese Beobachtung voll bestätigen. Nach einem 14tägigen Höbenaufenthalt im Forschungsinstitut Jungfrauoch (3450 m) musste ich zur Auslösung gleich starker subjektiver Störungen anstatt eines 5tägigen ein 7,5%iges CO-Luftgemisch während 15 Minuten in Ruhelage einatmen. Zwar waren hierbei Schrittpromen und Rechenaufgaben gleich gut möglich wie vorher nach Atmung von 1% CO mit 30%iger CO-Blutsättigung. Jedoch kollabierte ich 2mal beim Versuch frei herumzugehen. Dabei betrug mein Blut-CO-Gehalt 55% CO.

Man kann sich also innerhalb gewisser Grenzen an CO-Blut-Befall ähnlich wie an O₂-Mangel in einem gewisse Masse gewöhnen, was nach der neuen Erklärung, aber auch durch die Wirkung der 2,3-Phosphoglycerate einfacher zu deuten ist.

Die in der Literatur gelegentlich gemeldeten Fälle repetierter CO-Vergiftung mit nur 2% CO-Hb (z.B. Zorn) welche zu klinischen Erscheinungen führen, sind mit grosser Vorsicht aufzunehmen, wenn man sich an die Schwierigkeit genauer CO-Blutanalysen und an die Kriterien der korrekten Blutentnahmen erinnert und nicht zuletzt auch daran, dass die Symptome nach CO-Vergiftung sich noch erstärken können, zur Zeit da der CO-Gehalt im Blute schon wieder im deutlichen Abnehmen begriffen ist. Haldane weist auf S. 203 darauf hin und unsere Eigenversuche mit dem oft sekundär auftretenden Kopfschmerz nach der CO-Atmung weisen ebenfalls in diese Richtung. Auch hat Symansky (1965) man solle keinesfalls nur eine einzige CO-Bestimmungsmethode anwenden, da man bei keiner völlig sicher sei.

Übrigens sprechen unsere beschriebenen Versuche mit völlig symptomloser Einatmung von 4,5%iger CO-Luft (unreduziert) auf Jungfrauochhöhe mit einer Blut-CO-Sättigung von nur 12-15% CO gegen die Vorstellung einer Schädigung von nur 2% Blut-CO-Gehalt im Tal da diese 2% ja im obersten Teil der Haemoglobin-O₂-Sättigungskurve eintreten würden, die schon normalerweise nicht durch O₂ belegt sind.

Hyperventilationsversuch an CO-Merschweinchen

Unsere früher besprochene &

ergab sich in Bezug auf das erste Herumgehen dieser primär durch 20% CO-Luftgemisch komatös gewordenen Tiere, dass die kürzeste Erholungszeit in 20° Luft auftrat (19'45 als Durchschnittswert), die weitaus längste in 4° Luft (49'15") und eine mittlere in 37° Luft (28)

Als Erklärungsversuch hierfür sei folgende Annahme gemacht. Die komatösen CO-Tiere verhalten sich einer küsseren Abkühlung gegenüber wie hibernierte Warmblüter. Sie erleiden keine periphere Vasoconstriction und fühlen deshalb wie der narkotisierte Organismus ab. Dadurch aber wird der O₂ viel leichter an das Hb gebunden und folgedessen erschwert an das Gewebe abgegeben. Umgekehrt dürfte man sich vorstellen, dass durch Erwärmung der komatösen CO-Tiere deren O₂-Verbrauch gesteigert wird, wogegen die CO-Teilbelegung dieses Blutes eine zusätzliche O₂-Meherversorgung nicht zulässt, denn durch die benötigte Hyperpnoe geschieht die anfangs beschriebene alkalotische Erschwerung der cerebralen O₂-Verwertbarkeit.

Zur Frage der Vielgestaltigkeit der Symptome nach CO-Vergiftung

Der Satz von Ellinger (1931) wonach sich sämtl. e Autoren darüber einig seien, dass der Um- der Nachkrankheitssymptome in keinerlei Beziehung stehe zur Schwere der akuten Vergiftung, wird allerdings durch Teleky bezweifelt. Er fand, dass der Prozentsatz der Nachkrankheiten bei den Fällen, die mit langdauernder Bewusstlosigkeit begannen, viel grösser ist als bei den anderen.

Unter der bisher entwickelten Ansicht, dass es bei der menschlichen CO-Vergiftung keine spezifisch toxische CO-Wirkung gibt, kann die so grosse Mannigfaltigkeit der beobachteten Spätsymptome ausserordentlich vereinfacht werden bei Berücksichtigung der Tatsache, dass das ZNS das primär leidtragende Organ ist. Warum jedoch im einen Fall das Zuckerstoffwechselzentrum in einem andern das Vasomotorenzentrum in einem dritten das Temperaturregulationszentrum, in wieder andern mehr gestörte Funktionen betroffen und beruht meiner Ansicht nach auf der durchaus nicht immer einheitlichen Blutversorgung aller ZNS-Partien. Dies wissen die Neurochirurgen, seitdem man die cerebrale Angiographie macht und dies weiss besonders auch der Pathologe, der die verschiedenen Gefäss-Anomalien und Pathologien sieht.

Nach Kreuzer treten CO-Schädigungen vor allem in Hirngebieten mit relativ schlechter Kapillarkirkulation auf z.B. Globus pallidus rote Zone der Substantia nigra und an Oberflächen der Hirnrinde.

So mussten wir selbst einen 41jährigen Patienten begutachten welcher nach mehrfachem Kollabieren anlässlich von Gasleitungsreparaturen schlussendlich eine tiefe Bewusstlosigkeit davontrug. Dies führte zu einer über Monate anhaltenden Störung seines Vasomotorenzentrums als alleiniges Überbleibsel der CO-Vergiftung. Dieser Patient wies immer bald nach dem Aufstehen starken und progressiven Blutdrucksturz bei normaler Herzfähigkeit auf und wurde von der Verneuerung schlussendlich angenommen. Durch langes und systematisches Trainieren konnte das Vasomotorenzentrum wiederum errogen werden.

Ich möchte also diese Vielgestaltigkeit der nervösen Ausfälle auf die hypoxische Noxe des ZNS zurückführen. Damit aber vereinfacht sich wiederum die Ansicht über die schädliche CO-Wirkung beim Menschen.

Zur Diagnose leichter CO-Vergiftung

Durch unseren Hyperventilationstest im Liegen tritt schon bei 25%iger CO-Vergiftung, welche noch recht wenig Allgemeinsymptome macht, nach wenigen Sekunden ein kurzdauerndes Koma auf, welches in wenigen Sekunden spontan behoben ist und nach unseren zahlreichen Eigenversuchen wegen der Kürze seiner Dauer als durchaus harmlos bezeichnet werden darf. Dieser einfache Test soll die Blutanalyse nicht verdrängen, sondern im wesentlichen zur Bereicherung der Frühdiagnostik beitragen.

Zur Frage der chronischen CO-Vergiftung

In der Literatur besteht verschiedentlich die Ansicht, dass es eine chronische CO-Vergiftung gäbe als Folge einer langen Kette leichter „Angiftungen“, so besonders durch Symansky (1936) Drinker (1938) Zorn (1965) u. a.

Der Begriff der chronischen CO-Vergiftung sollte nur auf solche Fälle bezogen werden und nicht auf chronisch gewordene Schädigung durch einmalige starke CO-Vergiftung.

Mehrheitlich scheint nach dem internationalen Symposium der CO-Forschung von 1965 in Stuttgart die Meinung zu überwiegen, wonach es eine echte chronische CO-Vergiftung durch unter schwellige CO-Belegung des Blutes nicht gebe. Ny-

Punkt gestartet werden anstatt bei 25-50 oder 75% der CO-Vorlütigungswerte

Bei der menschlichen CO-Vergiftung dringt nun das CO vom oberen Ende der Blutsättigungskurve her ein, weil hier der Verdrängung des O₂ der geringste Widerstand entgegen gebracht wird. Das ist auch der Grund dafür, dass in der Höhe eine CO-Rohatmung im Liegen solange keine Symptome auslöst, als nur die oberste O₂-freie Partie der Blutsättigungskurve durch das CO beschlageneht wird. Erst bei weiterer CO-Sättigung treten dann progressives Austreiben von O₂ aus den Erythrocyten Symptome von Pulsbeschleunigung und späterer Dosigkeit auf.

Neu ist auch die Tatsache des rascheren Koma-eintrittes bei Hyperventilation in der Höhe als im Tale bei gleichen Graden von CO-Blutsättigungen. Dies wird auf einen spezifischen, funktionellen, cerebralen Alkaloseschaden bezogen. Dieser wird verstärkt durch die hyperventilatorisch starke Linksverschiebung der O₂-Blutbindungskurve, welche wiederum mit moderner spektrographischer Methode bestimmt nach oben progressive Linksverschiebung aufweist.

Diese neue Tatsache erklärt unsere Versuche, welche trotz konstantem Blutdruck bei stürkster Hyperventilation in kurzer Zeit zur Bewusstlosigkeit führten, zusammen mit der doch die Alkalose bedingten cerebralen Erschwerung der O₂-Verwertung.

Die neue Auffassung der herabgesetzten O₂-Spannung und Menge im CO-haltigen Blute erklärt viel besser die gewaltigen Unterschiede im Verhalten von Menschen mit Anämie von 50% Hb (welche noch arbeitsfähig sind) und bei 50%-iger CO-Blutsättigung, welche letzteren einen Zustand an der kausierten Grenze eines nur noch im Liegen erhaltenen Bewusstseins darstellt.

Es wird ein Eigenversuch mit 55%iger Blin-CO-Sättigung beschrieben, welcher zwar im Liegen zu zitteriger aber fehlerfreier Schrift führte, jedoch wiederholtes Kollabieren mit Bewusstseinsverlusten bei Geh-Versuchen bedingte.

Daneben wird durch Tierkurvenversuche (Kaninchen und Meerschweinchen) gezeigt, dass nicht das Herz, sondern zuerst das Vasomotorenzentrum und danach das Atemzentrum durch eine akute CO-Vergiftung gelähmt werden. Deshalb können solche im Schnellversuch bewusstlos gewordene und völlige Atemlahmung aufweisende Tiere durch sofortiges Einbringen in eine kleine

Überdruckkammer bei 2-3 Atm O₂-Druck in sehr kurzer Zeit wieder zum Bewusstsein gebracht werden, da auf diese Weise O₂ bei noch schlagendem Herzen in die Lungen gepresst wird und über den rein physikalischen Weg zum bald wiederum normal werdenden Leben genügt. Dabei helfen neben dem erheblichen O₂-Überdruck auch die während des längeren Atemstopps auftretende starke hypoxische Blutübersäuerung zum erstaunlich raschen Austreiben des CO. Deshalb können solche Tiere nach wenigen Minuten der O₂-Überdruckatmung wiederum symptomlos ausgeschleust werden.

Die höhere Empfindlichkeit kleinerer Säugetiere (Mäuse) gegenüber der CO-Vergiftung wird auf den erhöhten Grundumsatz kleinerer Tiere zurückgeführt. Interessant ist ferner die Tatsache, dass bei narkotisierten tracheotomierten Kleintieren (Kaninchen) die registrierten Atemkurven beim Übergang zu 5-10%iger CO-Atmung ohne vorherige Anzeichen unplotzlich slätern, wie dies auch nach i.v. Na-Nitrit Injektion geschieht.

Die klöuch so grosse Mannigfaltigkeit der Symptome nach einer CO-Vergiftung scheint nicht mehr so verworren, wenn man sich vorstellt, dass nicht alle Partien des ZNS gleichermassen durch Blut irriert werden. Da wo aus irgendeinem Grunde eine auch nur geringfügige Hypozirkulation vor besteht, wirkt sich der hypoxische Schaden eben am ersten und stärksten aus.

Unter Hyperventilationstest bei frischen fruglichen und leichten Fällen von CO-Vergiftung wird als neues einliches Hilfsmittel zur Früherfassung der Diagnose vorgeschlagen. Ein mögliches frühzeitiges Einbringen eines CO-vergifteten Menschen in 3 Atm O₂-Druck entspricht der bisher wirksamsten Therapie.

Am Schluss sei meiner treuen wissenschaftlichen Laborantin Frau L. Buchler herzlichst gedankt für ihre während nahezu 30 Jahren in vorbildlicher Art geleisteten Dienste.

LITERATUR

Gesamtüberblick über Literatur der CO-Vergiftung siehe im Internat. Symposium Kohlenmonoxyd-Innovationen Arbeitsmed. Soz. Med. und Arbeitshygiene, Heft I, 1965.

Ahlmark, A. u. Mitarb., Internat. Arch. f. Gewerbehyg. u. Gewerbehyg. 16, 13, 1957.

Barcroft, J. Philos. Trans. roy. Soc. London 211, 351, 1913.

Benesch, R., Benesch, R. u. Yassari, E. The action of hemoglobin and its extracts.

CO-Versuche ergaben jeweils den initialen Atemstop der bei nachfolgender Luftzufuhr allmählich unterbrochen wurde durch immer häufiger auftretende grosse Schnappatemzüge.

Es interessierte uns nun die Frage ob bei diesen vorarkotisierten CO-Tieren durch passive Hyperventilation gegen Luft auch die beim Menschen beschriebene schädliche Hyperventilations-alkalose-Wirkung zu erzielen wäre.

Die Resultate meines Dissertanden Strelt verheissen jedoch negativ - desgleichen auch wenn nach dem initialen CO-Atemstop eine verdünnte NaOH Lösung sofort i.v. nachgespritzt wurde. Damit scheinen narkotisierte Meerschweinchen welche übrigens gegen O₂-Mangel ohnehin schon viel resistenter sind als der Mensch diesen gefährlichen Effekt der Alkalosewirkung bei CO-Vergiftung nicht aufzuweisen.

Zur O₂-Therapie der CO-Vergiftung

Haldane und auch Henderson haben mit Recht auf die Wichtigkeit der CO₂-Zugabe zur therapeutischen O₂-Atmung bei CO-Patienten hingewiesen weil dadurch ein viel grösseres Atemvolumen und raschere CO-Austreibung erfolgen. Neuerdings schlug jedoch Kreuzer am internationalen CO-Symposium 1965 aus theoretischen

Gründen vor die O₂-Atmung bei CO-Vergiftung nicht mit einer CO₂-Atmung, sondern im Gegenteil mit einer Hyperventilation zu kombinieren was jedoch nach unseren beschriebenen Versuchen gefährlich sein kann wenn es nämlich im Verlaufe zur Hyperventilations-Alkalose kline.

Die eben beschriebenen Versuche mit der so raschen Erholungszeit akut CO-vergifteter Tiere durch O₂-Überdruck würden diese Therapie auch für den Menschen empfehlenswert erscheinen lassen. Jedoch würde dies nur für allererste Phasen der Vergiftung von grossem Wert sein also für Fälle welche noch nicht lange in tiefster Bewusstlosigkeit gelegen haben. Bei allen andern können unter Umständen hypoxische Schädigungen so weit vorgeschritten sein dass irreparable - vornehmlich cerebrale Ausfälle stattgefunden haben. Dies selbst in Fällen, wo im Blut (anlässlich der Späteleinweisung) dann nur noch Spuren von CO vorliegen, worauf Haldane schon hinwies. So gelang es uns nicht im Jahre 1945 eine CO-ergiftete Frau von 40 Jahren welche eine ganze Nacht lang in einem CO-vergasen Zimmer bewusstlos lag durch Einbringen in un-

sere O₂-Überdruckkammer mit 3 Atm O₂ zum Bewusstsein zu bringen. Wohl stieg der Blutdruck an doch starb die Patientin 12 Stunden nach der halbstündigen O₂-Überdruck-Behandlung ohne das Bewusstsein wiederum erlangt zu haben. Ein guter aber trauriger Beweis dafür dass unter der langen Hypoxie eben irreparable Schädigungen vornehmlich cerebraler Art stattfinden können.

Als Analogon hierzu bekam eine 60jährige Frau 4 Tage nach einer Struma maligna-Operation bei welcher ich die Carotis communis (die durch den Tumor umwachsen aber intakt war) erhalten konnte (und musste!) eine Spontanruptur dieser nicht mehr durch Tumor umwachsenen muskel schwach gewordenen Carotis. Nach bald erfolgter Blutersatz Infusion und Bluttransfusion in die rupturierte nicht mehr blutende Carotis gelang es zwar den Blutdruck wieder zu normalisieren, jedoch blieb die Patientin bewusstlos und starb nach 36 Stunden. Hier war das Gehirn ebenfalls irreversibel geschädigt. In gewisser Beziehung ein Analogon zum irreversiblen CO-Gehirnschaden.

1965 haben Goulon u. Mitarbeiter über 20 Fälle von CO-Vergifteten die in einer O₂-Überdruckkammer behandelt wurden berichtet. Diese Behandlung brachte in 13 Fällen komplette Heilung.

Zusammenfassung

Unsere frühere von vielen Klinikern begabte Ansicht einer spezifischen CO-Giftwirkung auf den Menschen veranlasste uns anhand der in der Literatur beschriebenen Hemmung der Carbohydrase durch CO an dieses Problem heranzutreten. Demnach dürften mittelstark CO-vergiftete Versuchspersonen bei Ruhehyperventilation keine oder stark verspätet auftretende Atmungstetanie aufweisen.

In 12 freiwilligen Versuchen mit jeweils 25-30%iger Blut-CO-Sättigung traten in Liegelage jedoch unerwarteter Weise schon nach 6-10 tiefsten und raschen Atemzügen kurze Bewusstlosigkeiten auf - Dies ist nach den Haldane'schen CO-O₂-Blut Sättigungskurven in Verbindung mit den durch CO₂-Verlust bedingten Bohrkurven nicht genügend zu erklären.

Zunächst wird auf eine irreführende Darstellung der CO-O₂-Blutbindungskurven im Buche von Haldane hingewiesen, wo trotz verschiedener Grade von CO-Vorsättigung die nachfolgenden O₂-Sättigungskurven immer bei Null auf dem gleichen

Haemodynamic studies at rest and during exercise in patients treated with artificial pacemaker

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- I. Effect of changes in ventricular rate on cardiac output and central pressures at rest and during exercise in patients with artificial pacemakers.
Beverlrd, S Jonsson, B Karlöf, I., Lagergren, H. and Sowton, E.
Cardiovasc. Res. 1 21, 1967
- II Effect of changes in ventricular rate on forearm bloodflow and systemic arterial pressure in patients with artificial pacemakers
Beverlrd, S Karlöf I and Resnekov L.
Scand. J clin Lab. Invest. 32 245 1973
- III. Adaptation of the left ventricle to sudden changes in heart rate in patients with artificial pacemakers.
Karlöf I., Beverlrd, S. and Överfors, C.-O
Cardiovasc. Res. 7 322, 1973
- IV Atrial-triggered pacemaking without thoracotomy Apparatus and results in twenty cases.
Lagergren, H., Johansson, L., Karlöf I and Thornander H.
Acta Chir Scand 132 678 1966.
- V Haemodynamic effect of atrial triggered versus fixed rate pacing, at rest and during exercise, in complete heart block.
Karlöf, I
Acta Med. Scand. 1974 In press.

References to these papers in the text will be made with the Roman numerals I—V

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Introduction

The treatment of syncope with a galvanic current was first suggested by Aldini in 1819 (2). Ten years earlier (1809) Burns had noticed that the heart could be stimulated by electricity (35). In 1932, Hyman published a report describing a pacemaker which was able to control the heart rate in experimental animals (21). He also suggested that it could be made portable and used in patients. Callaghan and Bigelow (1951) described successful experiments with electrical stimulation in the region of the sino-atrial node with a bipolar needle (7). The experiments were performed in animals with an intact conducting system. Zoll in 1952 was the first to successfully treat Adams-Stokes attacks with an artificial pacemaker (42). He applied electrodes externally to the chest wall. As this type of pacing needs high voltages, and therefore is painful, because of strong contractions in the muscles in the thoracic wall, attempts were made to use more direct stimulation. Thèvenet, Hodges and Lillehei 1958 stimulated the heart with a much lower impulse amplitude by means of an electrode introduced trans-thoracically (38). The threshold for electrical stimulation increased, however, rather soon because of formation of fibrous tissue around this electrode. The same year Furman and Robinson (17) reported that stimulation of the heart could be achieved with an electrode catheter introduced transvenously into the right ventricle. As a treatment for complete AV block which occurred during open heart surgery Weirich et al. (39) 1958 sutured electrodes on the myocardium and connected them to a small portable external pacemaker. In order to avoid wires passing out through the skin, with possibilities of infections following the cables to the heart, two fundamentally different types of implantable pacemakers were constructed. One was the inductive coupled pacemaker in which one coil was positioned subcutaneously and the other

superimposed externally and connected to the impulse generator (1, 8, 19, 34, 41). The other was the completely implantable pacemaker first developed by Elmqvist and implanted by Senning 1958 (16, 33). As energy source this unit had a pair of rechargeable, nickel-cadmium cells. In 1960 Chardack, Gage and Grestbach (12) described an implantable pacemaker with mercury cells, and since then, this has been the dominating energy source for pacemakers (15, 22, 28, 43). Implantable pacemakers, which usually give impulses at a fixed rate of around 70/min, have since then been improved throughout the years. In the beginning the impulses were transmitted to the heart with electrodes sutured to the epicardium, an operation which required thoracotomy. In order to avoid this major operation in patients who often were old and in a bad condition, Furman transvenously introduced an electrode catheter to the outflow region of the right ventricle (18). This method was further developed by Lagergren and Johansson who used an electrode with a very soft cable constructed by Siemens-Elema AB (24). This electrode was easily passed to the apical region of the right ventricle, the threshold for stimulation remained low and the cable did not break, a complication which was rather common with earlier electrodes.

In 1962 Nathan and Center implanted for the first time a pacemaker in which the impulses to the ventricles were triggered by the atrial activity (27). The P waves, picked up by an electrode sutured to the left atrium, triggered the pacemaker to give an impulse after an delay. In this way the pacemaker normal time relation between atrial and contraction, and the rate ~~differs~~ according to metabolic

A Swedish
constructed by S'

a patient for the first time in 1963 (10) The atrial electrode of this pacemaker was introduced by means of mediastinoscopy (9) In this way a patient could receive an atrial triggered pacemaker without running the risks of a thoracotomy

At about the same time many manufacturers started to construct pacemakers with the ability to sense the ventricular activity These were designed for patients with unstable AV-block, and have two principally different modes of function. One type is completely inhibited by the QRS complex, the other is triggered by the R wave to give a physiologically ineffective impulse in the QRS complex, as long as the patient is in sinus rhythm. In case of complete block, these pacemakers give impulses of a fixed rate to the right ventricle via the same electrode which senses the ventricular activity An R wave synchronous pacemaker was designed by Siemens-Elema AB in 1967 with a construction principally based on the atrial triggered pacemaker (23)

The haemodynamic effect of pacing at a fixed impulse-rate both at rest and during exercise has been studied by many authors (3, 4, 20, 26, 29, 30, 32, 36, 37) With the development of pacemakers with different modes of function the indications for the different types had to be further

analyzed. Was the haemodynamic benefit to a certain patient receiving an atrial triggered pacemaker so great that it could outweigh the possible disadvantages of giving him a more complicated and expensive unit? Or were there in some patients contraindications for one type of pacemaker? The beneficial haemodynamic effect of atrial triggered pacing has been emphasized among others by Braunwald et al. Brockman et al., Center et al. Martin et al., and Samet et al. (5, 6, 11, 23, 31) To the author's knowledge there is, however, no study in which the haemodynamic effect of atrial triggered versus fixed rate pacing has been investigated in the same patients during exercise on fairly high work loads.

The present study is in its first part an attempt to analyse firstly the haemodynamic effects of fixed rate pacing at different rates at rest and during work, secondly the immediate effect of sudden changes in rate. The second part of the study is devoted to atrial triggered pacing. The haemodynamic effects of a synchronized atrial systole per se as well as the effects of treatment with an atrial triggered versus a fixed rate pacemaker are analysed. Finally the indications and contraindications for treatment with atrial triggered pacemakers are discussed.

A Fixed rate ventricular pacing

L HAEMODYNAMIC STUDIES AT A CIRCULATORY RELATIVE STEADY STATE

Twenty-two patients with complete AV-block treated with artificial fixed rate pacemakers were studied in paper I. There were three women and nineteen men between the ages of 14 and 78 years, mean 52. Pacing was carried out with an intravenous electrode catheter previously placed in the apical region of the right ventricle (24) except in two patients, in whom epicardial electrodes were used (15). The indifferent electrode was positioned subcutaneously below the left costal arch. The cables were connected to an external pacemaker (Siemens-Elema, model EM 138) the impulse frequency of which could be varied between 30 and 170 impulses/min. The patients were investigated with heart catheterization in supine at different heart rates at rest and during exercise.

Effect of different pacing rates at rest

Cardiac output When the ventricular rate was changed from idioventricular to a normal resting level of about 70 beats/min, cardiac output increased, but then remained constant despite a further rate increase of up to about 160 beats/min.

Within the range of ordinary heart rates at rest, the cardiac output was normal or somewhat small in relation to the oxygen uptake. This means that the arteriovenous oxygen difference was normal or somewhat increased. In one patient who had mitral incompetence the arteriovenous oxygen difference was very high, even at normal heart rates.

Stroke volume The stroke volume was largest at idioventricular rate and decreased continuously with increasing ventricular rate.

Oxygen uptake When the ventricular rate was increased from an abnormally low to a normal level, the oxygen uptake did not change significantly.

Intracardiac and intravascular pressures The right ventricular filling pressure was abnormally high at very low ventricular rates, but decreased to normal levels when heart rate was increased. In the patient with associated mitral incompetence, the filling pressure, however, remained high despite increase in ventricular rate.

The pulmonary artery mean pressure was normal at low heart rate in most patients, and only slightly increased in a few. It remained almost unchanged at higher ventricular rates. The only exception was the patient with mitral incompetence in whom this pressure was moderately increased at low ventricular rates and showed a further marked increase at higher rates. The initially low aortic mean pressure was normalized, when the ventricular rate was altered from very slow to normal levels, around 70 beats/min. A further increase in rate caused an additional slight rise in pressure.

Peripheral vascular resistance did not change with variation of ventricular rate.

Effect of different pacing rates during exercise

In 15 patients the effect of exercise was studied at paced rates between 56 and 152 beats/min. During exercise at a fixed ventricular rate cardiac output increased in all patients, but was usually lower than normal in relation to oxygen uptake. Consequently stroke volume increased with increasing work load. In many patients there was a considerable rise in the right ventricular filling pressure and in pulmonary arterial pressure. The

aortic pressure increased normally in most patients.

Nine patients were exercised at levels close to their maximal capacity. At rates up to 110 beats/min, the stroke volume in most patients increased approximately to the value obtained at rest during idioventricular rhythm.

Three patients, who were exercised at rather high work loads at ventricular rates between 120 and 150 beats/min, were not able to attain their maximal stroke volumes. In these patients cardiac output did not increase further when the ventricular rate was raised above 110 beats/min.

In five patients the ventricular rate was altered between 56 and 109 beats/min, during exercise on a constant rather low work load. The stroke volume and right ventricular filling pressure decreased with increasing ventricular rate, and the cardiac output increased only slightly.

Conclusions

1. In patients with complete heart block treated with artificial unsynchronized ventricular pacing, cardiac output at rest increases when the ventricular rate is increased from slow idioventricular rate to 70 beats/min, but remains rather unchanged with a further increase in rate up to about 160 beats/min. During exercise cardiac output increases, in spite of a fixed ventricular rate, owing to an increase in stroke volume. Above a certain lower limit in ventricular rate the cardiac output is regulated by metabolic demands, relatively independent of further artificial variations in rate. This implies that no marked increase in cardiac output can be obtained by increasing ventricular rate in these patients neither at rest nor during work.

2. Above a certain lower rate, stroke volume varies inversely with ventricular rate. This is true both at rest, and during exercise on a constant work load.

Judging from the rather few patients studied during heavy work, a maximal stroke volume can be maintained up to a ventricular rate of the order of 110 beats/min. Above this rate stroke volume is decreasing.

3. At very low ventricular rates, aortic pressure

is low and the right ventricular filling pressure high. Both are more or less normalized when the ventricular rate is increased into the normal range of about 70 beats/min.

4. The investigation confirms that an implantable fixed rate pacemaker should give impulses at a rate around 70 impulses/min. If an implantable asynchronous pacemaker with variable frequency is constructed, its maximal rate could probably be around 110 impulses/min, if only an increase in cardiac output is intended.

II. HAEMODYNAMIC STUDIES OF SUDDEN CHANGES OF RATE

In paper I the responses of the aortic pressure and ventricular filling pressures, after a sudden increase of unsynchronized ventricular rate, were studied in 9 patients. The results obtained showed rather large, individual variations, but suggested that marked transient changes in blood flow may occur when a sudden change in ventricular rate is produced. Paper II is an attempt to get more information about the beat-to-beat adaptation of the circulation, following sudden changes in unsynchronized ventricular rate. The studies in this paper offer however only indirect information about the beat-to-beat changes in stroke volume. In paper III the sudden changes in stroke volume are studied more directly by measuring the angiocardiographic volume changes of the left ventricle, following sudden alterations in ventricular rate.

Effects on forearm blood flow forearm pulse volume and brachial artery pressure

The effect of sudden changes in heart rate at rest on forearm blood flow using strain-gauge plethymography and brachial arterial pressure, was studied in six patients being treated with external pacemakers for complete heart block (II). The age of the patients ranged from 55 to 77 years and none of them had any clinical or radiological evidence of cardiac decompensation at the time of the study. The amplification used for measuring forearm blood flow permitted increases in the forearm volume of each beat to be measured when

a plateau had been reached for each pulse. Ventricular rate was changed suddenly from a low idioventricular to a normal rate of 70 beats/min, from 70 to 150 beats/min and from slow idioventricular to 150 beats/min.

Immediate effects The forearm blood flow was relatively unchanged when ventricular rate was varied within broad limits, although sudden increase of rate, from idioventricular (27–49 beats/min) to a paced ventricular rate of 70 beats/min on the average, caused a minor but not significant increase in forearm blood flow. A sudden increase in ventricular rate was associated with a decrease in forearm pulse volume in all patients. Arterial pulse pressure likewise decreased in all but one patient, when ventricular rate was changed from idioventricular to 70 beats/min. An immediate decrease in ventricular rate was followed by immediate increases in forearm pulse volume and arterial pulse pressure. In each instance these changes in pulse-pressure amplitude and pulse volume occurred from one beat to the next, and appeared proportional to the diastolic filling time. Furthermore a beat-to-beat analysis showed a close correlation between changes in amplitude of the arterial pulse pressure and changes in forearm pulse volume.

Lat effects A further three to five measurements of forearm blood flow were made in a 90 second period, after changing ventricular rate. During this time when the rate was changed from slow idioventricular to 70 beats/min, transient increases in forearm blood flow were found in some patients, whereas flow stabilized within one minute on a slightly higher level. The difference was, however not statistically significant. In one patient whose idioventricular rate was rather high (42 beats/min) no change in flow was recorded.

When ventricular rate was changed suddenly from 70 to 150 beats/min, transient decreases in forearm blood flow occurred in four patients, but by the end of the 90 second period the flow had returned to approximately the same level as before the rate change. In one patient, however,

the flow was almost unchanged during the observation period.

When ventricular rate was changed from 30 to 150 beats/min, both transient increases and transient decreases in forearm blood flow were measured, but thereafter a new stable level of forearm blood flow was established.

Conclusions

1 Sudden changes in ventricular rate cause only minor and non-significant changes in forearm blood flow when measured immediately after and within approximately one minute from the change of rate.

2 Forearm pulse volume varies inversely with the ventricular rate in proportion to diastolic filling time, and the changes in pulse volume occur from one beat to the next. Presumably the changes in forearm pulse volume reflect changes in left ventricular stroke output.

Effects on left ventricular volume

Five patients, two women and three men, between the ages of 48 and 74 years were studied. All five patients had complete heart block of unknown origin. One of them also had an aortic stenosis (systolic pressure difference 50 mm Hg). They were treated with an external artificial pacemaker (Siemens-Elema, model EM 138) in which the impulse frequency could be varied between 30 and 150 impulses/min. The impulses were transmitted to the heart via a transvenous monopolar electrode, the tip of which was positioned in the apical region of the right ventricle. The patients were investigated in the supine body position. Aortic pressure was recorded through a catheter the tip of which was positioned in the ascending aorta. One plane cineangiocardiology was performed with injection of contrast medium in the right atrium, and exposures made at a rate of 42 frames/s in the right anterior oblique projection. During the passage of contrast through the left ventricle, the ventricular rate was suddenly altered by changing the impulse frequency of the pacemaker. The inner volume of the left ventricle was calculated regarding the left ventricle as a rota-

tional ellipsoid with concentric contractions. In one of the patients, who at the time of the study showed interference between sinus rhythm and pacemaker induced rhythm, no alteration in pacemaker rate was performed.

Stroke volume In patient no. 1 the ventricular rate was changed from an idioventricular rhythm of 45 beats/min to a pacemaker induced rate of 100 beats/min. The stroke volume calculated from the left ventricular volume changes decreased immediately from 135 to 75 ml. In spite of the decrease in stroke volume, the cardiac output calculated from the left ventricular volume changes and the heart rate increased slightly. At a circulatory steady state after 15 min with a heart rate of 100 beats/min, the cardiac output calculated from left ventricular volume variations was unchanged, compared with the cardiac output calculated at a ventricular rate of 45 beats/min. When the pacemaker was suddenly switched off in this patient, the heart rate fell instantaneously from 100 to an idioventricular rhythm of 27 beats/min, and stroke volume increased momentarily from 60 to 120 ml.

1) Patient no. 2 had initially an idioventricular rate of 35 beats/min when the pacemaker was suddenly switched on at a rate of 140 impulses/min. During the first beats, however the heart was stimulated only by every other impulse resulting in a heart rate of 70 beats/min. Approximately 15 min later the same procedure was repeated. This time the heart was activated by each pacemaker impulse, and the rate suddenly increased from 35 to 140 beats/min. At a change in heart rate from 35 to 70 beats/min, the duration of mechanical diastole at the higher rate still permitted unchanged diastolic filling of the ventricle during the first four beats. A twofold increase in heart rate and mainly constant stroke volume increased the calculated cardiac output from 3.5 l/min to 8.0 l/min, while the amplitude of the aortic pressure curve decreased somewhat. When the heart rate was suddenly changed from 35 to 140 beats/min, the stroke volume was reduced from one beat to the next to about one half giving a calculated cardiac output

of 7.7 l/min, which was of the same order as the cardiac output at a heart rate of 70 beats/min. In patient no. 3 the pacemaker induced ventricular rate was changed from 40 to 70 and from 70 to 100 beats/min, which gave an immediate change in stroke volume from 82 to 70 and from 67 to 50 ml respectively. In patient no. 5 the steps in rate were from 50 to 100 and from 100 to 50 beats/min giving immediate stroke volume changes from 90 to 60 and from 60 to 105 ml respectively.

This direct study of the left ventricular volume variations confirms the observations in papers I and II i.e. that stroke volume and pulse volume of the arm vary inversely with heart rate. It also confirms that the change in stroke volume occurs from one beat to the next following a sudden change in rate. This is, however true only within certain rate limits. It is obvious that in each subject there should be a lower limit for pulse rate, below which the heart can no longer compensate for the low rate by an increase in stroke volume, in order to keep cardiac output constant. When the heart rate is increased from a low level, stroke volume will initially remain unchanged up to a certain rate, where further shortening of the diastolic filling period will result in an inverse decrease of stroke volume with increase in heart rate. The level of this lower critical heart rate depends on such factors as myocardial compliance and available filling energy for the ventricles. This reasoning is exemplified by the study mentioned above in which an increase in ventricular rate from 35 to 70 beats/min caused no change in stroke volume during the first four beats recorded. According to the earlier studies, papers I and II, a decrease in stroke volume could be expected after these first beats probably depending upon a decrease of available filling energy due to decreased filling of capacitance vessels close to the heart. The transient increase of cardiac output, when heart rate is changed from a low to a normal level, is indirectly demonstrated by the repeated determinations of forearm blood flow (II).

End-diastolic and end systolic volume In the four patients mentioned above the alterations in

stroke volume were brought about by changes in end-diastolic ventricular volumes. The end-systolic ventricular volumes on the other hand were mainly constant in each patient, although the changes in heart rate and stroke volume were large. This was true also for patient no. 4, who at the time of the study showed interference between sinus rhythm and pacemaker induced rhythm. Here no alteration in pacemaker rate was performed. The study just shows ventricular volume changes during sinus beats and pacemaker induced beats. In spite of the different origin of the left ventricular activation, the volume changes were approximately the same, and the end systolic volumes mainly constant.

The principal factors determining the left ventricular end-diastolic volume seem to be the duration of mechanical diastole, the available filling energy during diastole and the elastic and resistive properties of the myocardium.

Conclusions

1 When heart rate is changed above a certain lower limit, left ventricular stroke volume varies inversely with heart rate.

2. The alterations in stroke volume are brought about by changes in left ventricular end-diastolic volume. These changes are proportional to the duration of mechanical diastole and filling energy. The end-systolic volume remains unchanged.

3 Left ventricular end-diastolic volume and stroke volume adapt, from one beat to the next, to changes in heart rate.

4. The results indicate that there is a lower border frequency at which the ventricular end diastolic volume is maximal. Below this frequency a further increase in length of diastole does not increase the stroke volume, and cardiac output then decreases in proportion to the decrease in heart rate.

B Atrial triggered ventricular pacing

I. Apparatus (IV)

Before the atrial triggered pacemaker could be constructed, the possibilities of deriving a P wave of sufficient amplitude from the atria and of using that P wave to trigger an artificial pacemaker, had to be studied. For this reason, an apparatus for cardiac resuscitation manufactured by Corbin Farnsworth Inc. Palo Alto Calif. was modified in 1963. After the modification the apparatus could give atrial triggered pacemaker impulses and the following parameters could be varied: input sensitivity, delay between input signal and impulse delivery, blocking time and basic rate. With this device the appropriate properties for an atrial triggered pacemaker were determined, and based on the collected data such a pacemaker was constructed by Siemens-Elema AB. In 1965 the first pacemaker was implanted in a patient. Since then the apparatus has undergone several stages of development, the principal data are, however unchanged. The current source comprises 4 mercury cells and the impulse voltage is about 5.4 V with a pulse duration of 2 ms (on later models decreased to about 1 ms). The pacemaker has a fixed basic rate of 50 impulses/min (on one model 60 impulses/min) and a highest synchronous rate of 150–170 impulses/min. It can be triggered by a P wave with a lowest amplitude of 0.9 mV (on a special model 0.4 mV) and the delay of the stimulating impulse is approximately 0 ms. The bandwidth of the P wave amplifier has been limited from 5 to 100 Hz in order to make the device less sensitive to external electro-magnetic interference. The output and input circuits have Zener diodes in order to decrease the risk of pacemaker damages, in case of external counter shock. When the atrial rate approaches the pacemaker's highest synchronous rate every 10th or 15th impulse drops out.

With a further rise in atrial rate, a constant 2:1 block appears.

II. IMPLANTATION OF THE ATRIAL DETECTOR ELECTRODE

P waves of an amplitude sufficient to trigger the pacemaker are obtained by an electrode positioned in close contact with the atria. This electrode is introduced as follows without thoracotomy by means of mediastinoscopy (9). Under general anaesthesia with the patient intubated, after administration of a muscular relaxant, a small incision is made in the jugular fossa. After perforation of the pretracheal fascia, dissection is performed by the aid of a finger along the trachea in the loose tissue to the front. The mediastinoscope is then introduced. Dissection is continued, under visual control, beyond the bifurcation of the trachea and behind the right branch of the pulmonary artery to the thin layer of connective tissue between the posterior wall of the atria and the oesophagus. The electrode tip is then placed, with the aid of a forceps, in the connective tissue as close as possible to the atrial wall. The amplitude of the P wave, led off by the electrode is checked on the electrocardiogram during the procedure. After a P wave amplitude of at least 1.0 mV has been obtained, the electrode is left in this position; the only fixation consists of suturing the cable in a few loops below the muscles of the neck. The rest of the cable is then passed subcutaneously to the right groin with the help of a wide-bore injection needle. Here the cable is passed out through the skin, in order to make it possible to check the amplitude of the P wave for some time during healing. The mediastinal electrode is of the same design as the electrode used for transvenous intra-ventricular pacing (21). It consists of a soft,

flexible cable, 1.5 m long and 1.2 mm in diameter. The cable has a core of Terylene around which four thin strips of fatigue-resistant stainless steel are wound. It has an insulating covering of polythene. A cylindrical electrode tip of platinum is attached to one end of the cable.

III. HAEMODYNAMIC STUDIES OF ATRIAL TRIGGERED PACING

Twenty-five patients with complete AV-block were studied using fixed rate and atrial triggered pacemakers (V). In all patients a transvenous electrode (Siemens-Elema, EMT 588) had previously been introduced to the apical region of the right ventricle, and an indifferent electrode had been placed in the fatty tissue of the abdominal wall. A *P* wave sensing electrode had been implanted by means of mediastinoscopy in each patient except one. In this patient the *P* wave was obtained from a temporary oesophageal electrode. The cables of the implanted electrodes were passed out through the skin in the right groin. The study consists of two series. In the first, including 12 patients, central pressures and cardiac output were measured at a fixed rate pacing of about 70 impulses/min, FRP and at atrial triggered pacing, ATP. Most patients were studied both at rest and during exercise, the work loads being identical with both types of pacemakers. In the second series 13 patients were studied at rest and during exercise. The measurements of pressure and cardiac output were first performed with the patients on ATP. After a 30 min rest an identical study was performed with the patient connected to a fixed rate pacemaker the rate of which was matched, FRPm, to the same as had previously been recorded with ATP.

This design of the investigation makes series I the practical clinical part of the study. The connection of the atrial triggered pacemaker implies, not only synchronization of atrial and ventricular activity but also a normal increase in heart rate during exercise as compared to the fixed pacemaker's asynchronous rate of about 70 impulses/min. Series II, on the other hand is an attempt to study the effect of the synchronous atrial systole

per se, the effect of rate being eliminated by adjusting the fixed rate pacemaker to the same rate as that obtained with the atrial triggered unit under the same conditions.

Effects of synchronized atrial systole

In series II, cardiac output with FRPm was on the average 4.5 l/min at rest, and increased during exercise to 8.5 l/min. The cardiac output under the same conditions with ATP averaged 5.3 l/min and 9.2 l/min respectively.

The heart rate with ATP averaged 77 beats/min at rest and 122 beats/min during work. With FRPm, interference rhythm occurred in three patients and the resulting heart rates were on the average 80 and 121 beats/min respectively. The resulting average stroke volume is significantly larger with ATP (69 ml) compared to FRPm (57 ml) at rest ($P < 0.01$). During work it is also larger with ATP but not significantly (77 ml compared to 72 ml). The filling pressure of the left ventricle, as reflected by the mean pulmonary artery wedge pressure or diastolic pulmonary artery pressure, was on the average lower with ATP significant on the 5% level at rest, whereas no difference was observed during exercise.

These data indicate that a normal timing of the atrial and ventricular contraction as obtained with ATP gave on the average an 18% higher cardiac output at rest and 8% higher during exercise compared to FRPm. It is somewhat remarkable that the effect of ATP on cardiac output is more marked at rest than during exercise. Furthermore the stroke volume is not significantly larger with ATP and there is no difference in filling pressure between ATP and FRPm during exercise. With increasing heart rate and shortening of diastole, the atrial contraction should have a greater importance than at rest for the diastolic filling of the ventricles. There can, however, be many factors explaining the moderate effect of ATP. One important factor can be that the work loads were not at all maximal on the average 409 kpm/min. This resulted in rather low average pulse-frequencies and conse-

quently the time for diastolic filling of the ventricles was rather long. Another factor is the marked individual variations. Some of the patients had an increase of cardiac output of more than 20 % with ATP compared to FRPm during exercise.

Effects of atrial triggered versus conventional fixed rate pacing on central haemodynamics

In series I, the effect of treatment with an atrial triggered pacemaker ATP compared to that of a fixed pacemaker at a rate of about 70 impulses/min, FRP, is studied both at rest and during exercise. The cardiac output with FRP was on the average 5.2 l/min at rest and increased to 8.2 l/min during exercise, at an average work load of 383 kpm/min. With ATP the average cardiac output was 5.7 l/min at rest and 9.8 l/min during work. Thus ATP gives a 10 % higher cardiac output ($P < 0.02$) at rest and a 20 % higher output during work ($P < 0.01$) compared to FRP. The heart rate with FRP was on the average 72 beats/min at rest and increased to 83 beats/min during work. This difference in average heart rate with FRP was caused by interference of normal, conducted sinus beats which was observed in three patients at rest and five during exercise. Because of the moderate increase in heart rate, the increase of cardiac output during exercise with FRP was mainly caused by a marked increase in stroke volume from 72 ml at rest to 104 ml during work. With ATP on the other hand, the increase of cardiac output during exercise was brought about primarily by an increase of heart rate from 83 beats/min at rest to 126 beats/min, and only to a minor degree by an increase of stroke volume, from 60 ml to 81 ml.

The filling pressure of the left ventricle as reflected by the mean pulmonary artery wedge (PCV) pressure or diastolic pulmonary artery pressure was on the average significantly lower ($P < 0.01$) with ATP than with FRP at rest, 9.2 and 12.5 mm Hg respectively. During work, there was no significant difference in filling pressures ($P > 0.05$) (23.9 mm Hg with ATP and 24.4 with FRP). As mentioned above, interference between fixed pace-

maker induced rhythm and conducted sinus rhythm occurred in five patients during exercise. When these five patients were excluded, the difference in cardiac output during work with ATP compared to FRP increased to 27 % and the PCV pressure was significantly lower with ATP on the 5 % level.

No patients in the study had a history of angina pectoris. In spite of that, two patients got angina pectoris during exercise when on ATP whereas on FRP they could perform the same work load without any discomfort. The signs of coronary insufficiency were obviously caused by increased myocardial oxygen consumption, when the heart was working at a higher rate and transporting more blood.

IV INDICATIONS FOR TREATMENT WITH ATRIAL TRIGGERED PACE MAKER

This presentation of the indications and contra indications for treatment with ATP is mainly based on findings presented in paper V and on subjective observations reported by patients treated with both fixed rate and atrial triggered pacemakers.

Most patients with a normal atrial activity and without coronary insufficiency feel better with ATP than with FRP. The cardiac output is higher and the ventricular filling pressure lower in most cases. Lack of coordination between atrial and ventricular systole, as with FRP results in a cyclic variation of the arterial pressure, which may be pronounced in patients with a low compliance of the ventricular wall, such as in aortic and pulmonary stenosis and in hypertrophic cardiomyopathies. In these patients the varying systemic blood pressure on FRP causes symptoms which vanish on ATP. The most important advantage with ATP is the ability to increase the ventricular rate during work. Young, and otherwise healthy patients, who are used to being physically active and therefore need a high oxygen transport capacity should be offered an ATP. In very old patients, the physical activity is generally not limited by the circulatory capacity and then an FRP satisfactory.

Patients who have an unstable AV-block, i.e. are changing between sinus rhythm and complete block, should have some type of triggered unit, as FRP in these patients gives rise to competition between sinus rhythm and pacemaker induced ventricular activity. This interference rhythm induces a risk for sudden death, because of the well known fact that an impulse from a fixed rate pacemaker falling in the vulnerable period (in the T wave) of the Ecg of a spontaneous beat can under certain but rare circumstances provoke a ventricular tachyarrhythmia, which can result in ventricular fibrillation. The risk for this complication must, however be small in most patients under normal conditions, as it was possible in the beginning of the pacemaker era to treat all patients, even those who most of the time were in sinus rhythm with fixed rate pacemakers, without incurring a high mortality. In materials from this time, varying incidents of sudden death have been reported. Sowton reported 1965 a five times higher incidence of sudden death in patients with competitive rhythm compared to those who followed the pacemaker without interference (37). Edhag, on the other hand, did not find a definite increase in death rate among patients with competition (13). Certainly these differences can be caused by differences in the materials. The fibrillation threshold could also be temporarily lowered in a patient, because of myocardial damage due to anaemia or electrolyte disturbance. This view is supported by observations made by Edhag on infarction patients in a cardiac care unit (14). He noticed that patients with coronary insufficiency and interference between pacemaker induced rhythm and sinus rhythm, had a higher incidence of ventricular tachyarrhythmias than patients with competition rhythm, but without coronary insufficiency. In a study of the vulnerable period Welti et al. (40) have reported two patients with recent myocardial infarctions and AV-block III, in whom each pacemaker impulse falling in the vulnerable period gave rise to ventricular fibrillation. The amplitude of the impulses in these two patients was just above the threshold for stimulation.

An argument for treating patients, who part of

the time have conducted sinus rhythm, with ATP is the possibility that they get a retrograde activation of the atria on FRP. The resulting rhythm has a very bad haemodynamic effect, as exemplified by a typical patient reported in papers IV and V. This patient had attacks of AV-block III, but was most of the time in sinus rhythm. The cardiac output at sinus rhythm was 6.1 l/min (HR 78 beats/min). When connected to the asynchronous pacemaker (HR 88 beats/min) the cardiac output decreased to 4.3 l/min, and the filling pressures of right and left ventricles rose markedly. When an atrial synchronous pacemaker was connected (HR 78 beats/min) the cardiac output increased again to 6.3 l/min and the filling pressures decreased. The explanation of the very marked decrease in cardiac output and stroke volume with FRP was retrograde activation of the atria, which, instead of contributing to the filling of the ventricles in end-diastole, regularly contracted against closed atrio-ventricular valves and emptied in a retrograde direction into the big veins. This retrograde pumping of the atria is also often noticed by the patients as an unpleasant sensation in the chest.

For patients who most of the time have conducted sinus rhythm, some type of entricular triggered (R wave inhibited or R wave synchronous) pacemaker is the type of choice. Some of these patients, however use to get complete block regularly when performing heavier exercise. It is obvious that this implies an indication for the somewhat more complicated treatment with ATP. In a discussion about patients with unstable complete AV-block, it has to be pointed out that this diagnosis cannot be made until the pacemaker treatment has started. It is a common observation that in quite a few patients, who before treatment have had a stable complete AV-block III for a long time, the heart starts to interfere with conducted sinus rhythm, when a pacemaker has been connected for some time. Because of this, a patient who is about to get his first pacemaker implanted should either have some type of triggered unit or should first be treated for some time with an external pacemaker.

V CONTRAINDICATIONS FOR TREATMENT WITH ATRIAL TRIGGERED PACEMAKER

Patients who have or have had atrial arrhythmias, such as atrial fibrillation or flutter, sinus bradycardia or sinus arrest, should not be treated with ATP. If however a patient treated with ATP gets atrial flutter or fibrillation this does not usually disturb him too much. The electrical amplitude led off from fibrillating atria is usually too low to trigger the pacemaker, which because of this gives impulses at its basic rate of around 50 impulses/min. The amplitude of atrial flutter usually is high enough to trigger the pacemaker but as the resulting impulse rate due to the construction of the device is generally around 100–130 impulses/min, the patient only feels a minor discomfort.

Coronary insufficiency is a contraindication against treatment with ATP. The physical activity in patients with ischemic heart disease is limited by the chest pain which will occur at a lower work load at ATP because of the higher heart rate and myocardial oxygen consumption as compared with FRP. It is also a common clinical observation that these patients have a better exercise tolerance with FRP than with ATP.

A relative contraindication for treatment with ATP can be that the patient has an occupation, in which he is exposed to strong electromagnetic interference, which could trigger the rather sensitive pacemaker. Such external electromagnetic disturbance of the pacemaker's activity does not imply a life risk, but is unpleasant for the patient.

Conclusions

Indications for ATP

- 1 AV-block III in physically active patients with normal atrial activity
- 2 Patients with retrograde activation of the atria on FRP
- 3 Patients in sinus rhythm at rest who get AV block III during exercise
- 4 Some patients with low compliant myocardi, such as aortic and pulmonary stenosis and hypertrophic cardiomyopathies, where the atrial contraction is essential for the necessary filling pressure

Contraindications for ATP

- 1 Atrial arrhythmias such as atrial flutter and fibrillation, sinus bradycardia, sinus arrest.
- 2 Ischemic heart disease.
- 3 Occupational risks of frequent electromagnetic disturbance of the pacemaker

General summary

The present investigations were planned to deal with the influence of heart rate on central haemodynamics, left ventricular performance and peripheral blood flow in patients with acquired complete heart block. Pacing of the heart was induced in the right ventricle. Heart rate was varied either artificially by alteration of impulse frequency of a fixed rate pacemaker or in the case of an atrial triggered pacemaker by the spontaneous sinus rate of the patient. Studies were performed both at rest and during work, in steady state conditions. These measurements were supplemented by studies of the effect of sudden changes in heart rate on peripheral blood flow, blood pressure and left ventricular performance.

Fifty-three patients suffering from AV-block III were studied. The mean age of the patients is lower than that generally observed in pacemaker patients.

In the course of the work, a new pacemaker was developed which allowed the ventricular rate to be controlled by the atrial electrical activity with normal time relation between atrial and ventricular contraction. The ventricles were paced by means of a transvenously introduced electrode, the tip of which was positioned in the apical region of the right ventricle. The atrial impulse was sensed with an electrode of the same design positioned by means of mediastinoscopy close to the atrial wall, where a P wave amplitude of more than 1 mV could be obtained.

Exercise was performed in supine on a bicycle ergometer. Central haemodynamics were studied by means of heart catheterisation with measurement of pressures and flow. Regional blood flow was measured with venous occlusion plethysmography and left ventricular volume was calculated from cine films, which were taken after a right atrial injection of contrast medium.

The effect of varying the rate of right ventricular pacing was first studied at rest in 22 patients. At idioventricular rhythm with a mean heart rate of 34 beats/min, the circulation was markedly hypokinetic, and increasing the rate by means of the fixed rate pacemaker resulted in an increase of cardiac output, without a change of oxygen uptake shifting the kinetics of the circulation to a more normal state. Further increases in heart rate, up to rates between 132 and 166 beats/min, resulted in only minor changes in cardiac output. Stroke volume at rest was close to its maximum at idioventricular rhythm and decreased when artificial pacing was initiated. Within the range of pacing rates, where cardiac output remained more or less constant, stroke volume varied inversely with heart rate. The filling pressure of the right ventricle, measured as right atrial mean or right ventricular end-diastolic pressure, varied with stroke volume, i.e. decreased with increasing heart rate.

The effect of varying rate during exercise only dealt with studies where the artificial pacemaker was used. With exercise at a fixed ventricular rate, the cardiac output increased in all patients, but was usually lower than normal in relation to oxygen uptake. Consequently the stroke volume increased in proportion to the work load. At a constant work load, the stroke volume and right ventricular filling pressure decreased with increasing ventricular rates, and the cardiac output increased only slightly. The latter study was performed at ventricular rates between 55 and 110 beats/min.

It is concluded that above a certain lower heart rate level, cardiac output is determined by the metabolic activity of the body and not by heart rate per se. The fact that the cardiac output is constant implies that stroke volume varies inversely with heart rate. The filling pressures during exercise

were higher than at rest, but decreased with increasing rate.

With the aim to study the effect of sudden changes of heart rate, forearm blood flow was recorded with venous occlusion plethysmography in 6 patients at rest in supine body position. Heart rate was changed over a wide range (30—150 beats/min). The plethysmography curve allowed beat-to-beat forearm pulse volume changes to be determined. Arterial blood pressure was measured via a catheter in the brachial artery of the opposite arm.

Sudden increases or decreases in heart rate did not affect forearm blood flow to any significant extent. Forearm pulse volume and arterial pulse pressure varied inversely with heart rate. These changes in pulse volume and pulse pressure occurred from one beat to the next.

Assuming that variations in forearm blood flow reflected variations in cardiac output, and that changes in pulse volume and arterial pulse pressure reflected changes in stroke volume of the heart, it was concluded that sudden changes in heart rate do not change cardiac output to any significant extent, and that stroke volume decreases with increasing heart rate. These changes occur from one beat to the next when heart rate is changed stepwise.

The cineangiographic volume studies were performed at rest in supine position. Heart rate was changed stepwise by means of an external pacemaker while contrast medium was passing through the left ventricle, thus allowing a beat-to-beat study of the volume changes. A sudden increase in rate resulted in a decrease in stroke volume from one beat to the next. This decrease was effectuated by a decrease in the end-diastolic volume of the left ventricle while the end-systolic volume remained unchanged. A transient increase of the calculated cardiac output was observed when heart rate was increased within certain limits. The magnitude of this increase in cardiac output was related to whether the control level of the heart rate was subnormal or not. A decrease in heart rate resulted in an increase of the stroke volume and of the end-diastolic volume of the heart.

The importance of a properly timed atrial systole for central haemodynamics was studied in patients at rest and during exercise, paced alternatively with atrial triggered and with fixed pacing. At approximately the same rate as that obtained with atrial triggered pacemaking. With this experimental design, all other variables were assumed to remain unchanged.

At the same oxygen uptake at rest and during exercise, cardiac output was on the average 0.75 l/min higher with atrial triggered pacing. At atrial triggered pacing this increase was effectuated by an increase in stroke volume, which was larger at rest than during exercise.

To evaluate the importance of an increasing heart rate for central haemodynamics in patients with complete AV-block, studies were made at rest and during exercise at the same load with atrial triggered pacing and fixed pacing at a rate of about 70 impulses/min. With this experimental design, the combined effect of the synchronized atrial contraction and the spontaneous increase in rate was compared to fixed rate pacing. With fixed rate pacing, the circulation was slightly hypokinetic at rest and more so during exercise. The increase in cardiac output during work was accomplished by large increases in stroke volume and by increases in ventricular filling pressure. With atrial triggered pacing at the same oxygen uptake the circulation was less hypokinetic and the increase of stroke volume less marked. In spite of the higher cardiac output, ventricular filling pressure was the same or somewhat lower. Thus, with atrial triggered pacing, a larger cardiac output may be obtained with a lower ventricular filling pressure.

GENERAL CONCLUSIONS

At fixed rate ventricular pacing metabolic activity is the main determinant of cardiac output above a certain lower limit of heart rate both at rest and during exercise.

Stroke volume and filling pressures vary inversely with heart rate above this critical rate, and cardiac output remains relatively constant.

Sudden changes in heart rate are met by changes

in stroke volume from one beat to the next, decreasing with increasing heart rate.

This decrease in stroke volume is effectuated by a decrease in end-diastolic ventricular volume, while the end systolic volume remains unchanged.

Normal timing of atrial and ventricular con-

traction results in measurable increases in cardiac output compared to asynchronous activity

Atrial triggered pacing, when applicable, is a superior treatment of complete AV-block, allowing a more normokinetic circulation during exercise compared to fixed rate pacing.

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References

- 1 Abrams, L. D. Hudson, W. A. & Lightwood, R. A surgical approach to the management of heart block, using an inductive coupled artificial cardiac pacemaker *Lancet* 1 1372, 1960.
- 2 Aldini, G. General views on the application of galvanism to medical purposes principally in cases of suspended animation. London, J. Callow 96, 1819
- 3 Benichou, A., Li, Y. B. & Diamond, H. G. Cardiovascular dynamics in complete heart block at various heart rates. (Effect of exercise at fixed heart rate) *Circulation* 30: 342, 1964.
- 4 Bentivoglio, L. G. Geary M., Menendez, M., Schlemmer, Z. & Goldberg, H. Cardiovascular dynamics in man during idioventricular rhythm and at various, electrically determined, ventricular rates in acquired complete heart block at rest. *Cardiologia* 34 170, 1969
- 5 Brunsfeld, E. & Fraxen, C. J. Studies on Starling law of the heart IV Observations on the hemodynamic functions of the left ventricle in man *Circulation* 24 633 1961
- 6 Brockman, S. K. Collins, H. A. Bloomfield, D. A. Sinclair Smith, B. C. & Gobbel, W. G. Physiological studies and clinical experience in patients with synchronous and asynchronous pacemakers *J Thoracic Cardiovasc. Surg.* 51 864, 1965
- 7 Callaghan, J. C. & Bigelow, W. G. An electrical artificial pacemaker for standstill of the heart. *Ann. Surg.* 134 8, 1951
- 8 Camerilli, L., Pozzi, R. Drago, G. Pizzichini, G. & De Saint Pierre, G. Stimolazione elettrica del cuore nelle sindrome di Morgagni-Adams-Stokes mediante trasmettitore radio frequenza bobina ricevente epicardica (prima applicazione clinica) *Osped. Ital. Chir.* 5 999 1961
- 9 Carls, E. Mediastinoscopy: A method for inspection and tissue biopsy in the superior mediastinum. *Dis. Chest* 36 343, 1959
- 10 Carls, E., Johansson, L., Karlöf, I. & Lagergren, H. New method for atrial-triggered pacemaker treatment without thoracotomy *J Thoracic Cardiovasc. Surg.* 50 229 1965
- 11 Cencer, S., Nathan, D. W., C. Y. & Doque, H. Two years of clinical experience with the synchronous pacer *J Thoracic & Cardiovasc. Surg.* 48 513 1964
- 12 Chardack, W. Gage, A. & Greenhatch, W. A transistored, self-contained, implantable pacemaker for the long-term correction of complete heart block. *Surgery* 48 643, 1960.
- 13 Edhag, O. Long term cardiac pacing. Experience of fixed rate pacing with an endocardial electrode in 260 patients. *Acta Med. Scand. Suppl.* 302, 1969
- 14 Edhag, O. Personal communication.
- 15 Elmquist, R. Landegren, J. Pettersson, S. & Sennings, A. & William-Olsson, G. Artificial pacemaker for treatment of Adams-Stokes syndrome and slow heart rate. *Ann. Heart J.* 10 731 1963
- 16 Elmquist, R. & Sennings, A. An implantable pacemaker for the heart *Medical Electronics Ind. Int. Conf. Peru* 1959
- 17 Fernan, S. & Robinson, G. The use of an intracardiac pacemaker in the correction of total heart block. *Surg. Forum* 9 245 1958
- 18 Fernan, S. Schrevel, J. Robinson, G. & Herwitz, E. Use of an intracardiac pacemaker in the control of heart block. *Surgery* 49-98, 1961
- 19 Glenn, W. W. L., Mauro, A. Longo, E., Lavietes, P. H. & MacKay, F. J. Remote stimulation of the heart by radiofrequency transmission. Clinical application to patients with Stokes-Adams syndrome. *New Engl. J. Med.* 261 948, 1959
- 20 Hempt, G. J. Myers, R. N. Daly, J. W. & Birkhead, N. C. Implanted cardiac pacemakers of variable frequency (hemodynamic studies of two patients) *J. Am. med. Ass.* 185 87 1963
- 21 Hyman, A. S. Resuscitation of the stopped heart by intracardiac therapy *Arch. Int. Med.* 50. 283 1932.
- 22 Kastrowitz, A., Cohen, R. Raffard, H., Schmidt, J. & Feldman, D. S. The treatment of complete heart block with an implantable, controllable pacemaker *Surg. Gynec. and Obst.* 115 417 1962.
- 23 Karlöf, I., Lagergren, H. & Thorenander, H. Ventricular triggered pacemaking without thoracotomy Apparatus and results in twenty cases. *Scand. J. Thoracic Cardiovasc. Surg.* 2 105 1968.
- 24 Lagergren, H. & Johansson, L. Intracardiac stimulation for complete heart block *Acta Chir. Scand.* 125 562, 1963.
- 25 Martla, R. H. & Cobb, L. A. Observations on the effect of atrial systole in man *J. Lab. & Clin. Med.* 68. 224, 1966.
- 26 Müller, O. F. & Bellet, S. Treatment of intractable heart failure in the presence of complete atrio-ventricular

- tricular heart block by the use of the internal cardiac pacemaker *New Engl. J Med.* 263 768, 1961
- 27 Nathan, D A., Center, S Wu, C Y & Keller W An implantable, synchronous pacemaker for long-term correction of complete heart block. *Circulation* 27 682 1963
 - 28 Portal, R W Davies, J G Lenthall, A. & Siddons, A. H M Artificial pacing for heart block. *Lancet* 2 1369 1962.
 - 29 Samet, P Cardiac pacing Grune & Stratton, Inc. International standard book number 0-8089-0800-6, 1975
 - 30 Samet, P Bernstein, W H., Medow A. & Nathan, D A Effect of alterations in ventricular rate on cardiac output in complete heart block. *Amer. J Cardiol.* 14 477 1964
 - 31 Samet, P Bernstein, W Nathan, D A. & Lopez, A. Atrial contribution to cardiac output in complete heart block *Am. J Cardiol.* 16 1 1965
 - 32 Segel, N Hadson, W A., Harris, P & Bishop, J M The circulatory effects of electrically induced changes in ventricular rate at rest and during exercise in complete heart block. *J clin. Invest.* 43 1541 1964.
 - 33 Senning, A Discussion of paper by Stephenson et al. *J Thoracic Surg.* 38 639 1959
 - 34 Siddons, A. H. M Long term artificial cardiac pacing: experience in adults with heart block *Am Roy Coll. Surg. Engl* 33 22, 1963.
 - 35 Siddons, A. H M & Sowton, E. Cardiac Pacemakers, Charles C. Thomas Publishing Company Ill USA. 1967
 - 36 Sowton, E. Haemodynamic studies in patients with artificial pacemakers. *Brit. Heart J* 26 737 1964.
 - 37 Sowton, E. Artificial pacemaking and sinus rhythm *Brit. Heart J* 27 311 1965
 - 38 Thevenet, A. Hodges, P C. & Lillehei, C W The use of a myocardial electrode inserted percutaneously for control of complete sino-ventricular block by an artificial pacemaker *Dis. Chest* 54 621 1958.
 - 39 Weirich, W L., Paneth, M., Gott, V L. & Lillehei, W C. Control of complete heart block by use of an artificial pacemaker and a myocardial electrode. *Circulation Research* 6 410, 1958
 - 40 Welti, J J Fontaine, G & Facquet, J Période vulnérable: risques possibles des rythmes inter-séquentiels et des stimulations pairées ou complètes chez l'homme *Arch. Mal. Coeur* 60 502 1967
 - 41 Vermeulen, H Webb, R. G J & Kelly M Radio control of ventricular contraction in experimental heart block. *Science* 128 1003, 1958.
 - 42 Zoll, P M. Resuscitation of the heart in ventricular standstill by external electric stimulation. *New Engl J Med* 157 768, 1952.
 - 43 Zoll, P Frank, H Zaruly L., Lownthal, A. & Bergard, A Long term electric stimulation of the heart for Stokes-Adams disease *Am Surg* 134 330, 1961

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Studies on an Intravenous Fat Tolerance Test

Methodological, Experimental and
Clinical Experiences with Intralipid®

By Stephan Rossner

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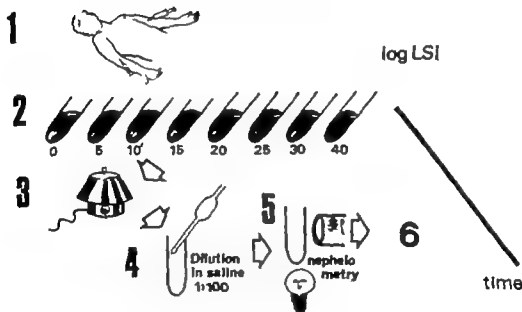
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- III L.A. Carlson L. Karjaer S Rössner and M.L. Wahlqvist Myocardial metabolism of exogenous plasma triglycerides in resting man. *Acta med scand* 193 233 1973
- IV Ulla Freyachius, D Hallberg L. Johnson and S Rössner Removal of exogenous plasma triglycerides in splanchnic viscera

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 - VI S Rössner U Larsson-Cohn, L.A. Carlson and J Boberg Effects of an oral contraceptive agent on plasma lipids, plasma lipoproteins, the intravenous fat tolerance and postheparin lipoprotein lipase activity *Acta med scand* 190 301 1971
 - VII S Rössner and U Rosenqvist Serum lipoproteins and the intravenous fat tolerance test in hypothyroid patients before and during substitution therapy Accepted for publication in *Atherosclerosis*
- These publications will be referred to in the text by their Roman numerals

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INTRODUCTION

Elevated concentrations of blood lipids are now commonly accepted as risk factors for the development of atherosclerotic diseases. This holds true not only for hypercholesterolaemia but has also been found to be valid for hypertriglyceridaemia (1, 2, 3). The rapid development within the field of atherosclerosis research has broadened our present knowledge about the relation of elevated blood lipids to atherosclerotic diseases since the introduction of the lipoprotein concept (4). These lipoproteins have been classified by various methodological approaches such as ultracentrifugation, electrophoresis or nephelometry (5, 6, 7, 8). One of the most useful techniques to separate lipoproteins is to use the preparative ultracentrifuge. With this technique three major lipoprotein classes are isolated: Very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) and in these fractions TG and cholesterol can subsequently be determined (8).

Following the absorption of dietary fat chylomicrons are formed. These particles contain mainly TG and are rapidly removed from the circulation. After an overnight fast chylomicrons are normally not present in the blood. VLDL are mainly synthesised in the liver. In the fasting state most of the circulating TG are carried in the VLDL, which also have a rapid turnover rate. In VLDL a small part of the circulating plasma cholesterol is also found. However, the main part of the circulating cholesterol is transported in the LDL fraction which generally carries only a small proportion of TG. The HDL fraction contains mostly cholesterol and phospholipids and only little TG. This fraction may however play an important role in the transport of VLDL TG in the circulation. The function of the plasma lipoproteins has recently been reviewed by Robinson (9).

Hypertriglyceridaemia is the result either of an increased secretion of TG into the circulation or of an impaired removal. These processes may also be combined. During recent years evidence has accumulated that hypertriglyceridaemia is caused by a removal defect rather

than by an increased secretion of TG into the circulation (10). It is therefore natural that various methods have been developed to study the elimination of plasma TG from the blood stream.

The first methods used for this purpose were oral fat loads of different kinds (11). In these tolerance tests fats of different kinds were administered orally. After a standardised fatty meal the turbidity of blood plasma was used as a measurement of the TG concentration. Tracers such as labelled fats, vitamin A etc. were also administered and the concentration of these tracers in blood plasma was determined by isotopic TG analysis, vitamin A measurements etc.

Oral tests were found to have disadvantages, because it was difficult to evaluate the time course for exogenous plasma TG removal from the blood stream for the following reasons. The blood levels during such tests depend not only on the rate of disappearance of exogenous TG from the blood stream but also on various absorption processes in the gastro-intestinal tract: the formation rate of chylomicrons in the gastro-intestinal tract, the influx into the circulation, the possible appearance of "endogenous" TG in the circulation after the removal of chylomicrons etc. The shape of such TG elimination curves may thus depend on several complex processes and be difficult to interpret.

In the following discussion exogenous lipids are used as a term for lipids in chylomicrons entering the blood stream from the thoracic duct during absorption of dietary fat or lipids in artificial fat emulsions given intravenously. If these lipids reappear in plasma after once having left the blood stream, they are called endogenous lipids. Endogenous plasma TG may also be newly synthesized.

Several methods have been introduced to bypass the influence of the gastrointestinal tract on TG resorption and chylomicron formation. A physiological way to determine the removal of exogenous TG from the blood stream is to study the elimination of intravenously injected chylomicrons. Although such studies have been carried out in both experimental animals and in

man (12-13) it is not possible for several practical reasons to use such a method as a clinical routine test. Interest has therefore been focused on fat emulsions that can serve as substitute for chylomicrons. For such a test to be valid the fat emulsion must serve as a tracer of chylomicron metabolism. Fat emulsions of different composition or with isotopically labelled TG have therefore frequently been used (14-15). Direct uptake of chylomicron TG by liver, skeletal muscle and adipose tissue as well as lipolysis have been mechanisms believed to result in chylomicron TG clearance from blood (9). Many artificial fat emulsions however seem to be taken up by the reticuloendothelial system to such an extent that they cannot be regarded as tracers of chylomicron metabolism (16).

The fat emulsion Intralipid® (Vitrum AB, Stockholm, Sweden) has been found to have excellent clinical tolerance (17). Intralipid® resembles chylomicrons in several aspects. They have similar enzyme kinetic behaviour as substrate *in vitro* with post-heparin lipase and the kinetics for the elimination of Intralipid® and chylomicrons are identical in dog and in man (12-18-19). There are thus several reasons why Intralipid® may be a valid tracer for chylomicron metabolism.

In 1963 Carlson and Hallberg demonstrated that after an intravenous single dose injection of Intralipid® the disappearance of the emulsion from the blood stream could be characterized by two rate constants (12). At high Intralipid® concentrations a maximal removal and zero order reaction rate (k_1 $\mu\text{mol}/\text{min}$) was found and below a critical concentration the removal rate was of the first order (k_2 $\%/ \text{min}$). Based on these findings Boberg, Carlson and Hallberg introduced an intravenous fat tolerance test (IVFTT) 1969 using the Intralipid® emulsion and determining k_2 (10). k_1 and k_2 were not related to each other nor were k_1 and the plasma TG concentration in this study. However, k_2 was found to correlate with the plasma TG concentration in a reciprocal way so that subjects with high k_2 values had low plasma TG concentrations and vice versa.

The negative relationship between the IVFTT and the serum TG concentration has been analyzed in several clinical studies (21-22-23-24-25). However, in order to obtain data on k_2 characteristics also from healthy subjects with normal serum lipid levels and to compare these with the corresponding values obtained from a group of individuals with asymptomatic hyperlipoproteinaemia, a population study has been carried out (26). This study was performed on apparently healthy subjects attending a health control centre. The hyperlipoproteinaemic subjects participating in the investigation were active professional people who at the health control centre incidentally were found to have elevated serum lipids. They comprised about the upper 3 per cent of the population analysed and were referred to the Lipid Unit, Karolinska Hospital. At the Lipid Unit a detailed medical history was taken and an extensive clinical and laboratory examination was carried out. Subjects who had a history of atherosclerotic manifestations or of secondary hyperlipoproteinaemia or were treated with drugs expected to affect lipid metabolism were excluded. Some of the subjects classified as hyperlipoproteinaemic during the screening procedure had "normal" lipid values when investigated at the Lipid Unit. These subjects were also included in the study and referred to as "normals". Matched controls were selected from the same health control centre. The controls were of the same age and sex as the corresponding hyperlipoproteinaemic subjects, but had a serum TG concentration below 2 mmol/l and a serum cholesterol concentration below 270 mg/100 ml on at least three occasions. Fasting serum lipids and the IVFTT k_2 value were determined as described (27-28 study I). The sera of the subjects were typed according to a WHO report (29). Clinical data and results from this study are summarised in Table I and the relationships between k_2 and serum TG in the different groups are given in Fig. 1.

In earlier studies on the negative relationship between k_2 and fasting TG concentration there has been an impression that this relation rather

Table I Clinical data: total serum TG and cholesterol on the IVFTT occasion in control and hyperlipoproteinaemic subjects (ref. 26). Mean \pm SEM are given.

	n	Age years	TG mmol/l	Chol mg/100 ml	Weight/Height index kg/cm ² 100	IVFTT k ₂ %/min	IVFTT k ₂ range
Control subjects	M 45 F 43	48 \pm 2 53 \pm 1	1.01 \pm 0.05 1.03 \pm 0.04	222 \pm 5 234 \pm 6	0.96 \pm 0.02 0.94 \pm 0.01	6.09 \pm 0.41 7.15 \pm 0.33	2.21-19.88 4.21-11.22
	"Normals"						
Hyper- lipopro- teinaemic subjects	M 27 F 24	48 \pm 2 54 \pm 2	1.91 \pm 0.22 1.75 \pm 0.13	263 \pm 8 302 \pm 8	0.99 \pm 0.03 0.99 \pm 0.03	3.82 \pm 0.31 5.05 \pm 0.45	1.74-8.71 2.11-11.32
	Type IIa						
	M 17 F 37	49 \pm 2 56 \pm 1	1.68 \pm 0.09 1.79 \pm 0.10	350 \pm 10 351 \pm 9	0.98 \pm 0.02 0.98 \pm 0.02	4.30 \pm 0.31 5.21 \pm 0.31	2.41-8.16 2.26-9.34
	Type IIb						
	M 15 F 12	52 \pm 2 56 \pm 2	2.57 \pm 0.27 3.02 \pm 0.24	340 \pm 12 349 \pm 14	1.03 \pm 0.02 1.02 \pm 0.04	2.89 \pm 0.15 3.58 \pm 0.25	2.05-3.88 1.91-4.73
	Type III						
	M 12 F 6	48 \pm 3 55 \pm 3	3.73 \pm 0.79 3.21 \pm 0.13	296 \pm 17 335 \pm 31	0.99 \pm 0.03 1.08 \pm 0.09	3.43 \pm 0.41 4.16 \pm 0.52	1.83-6.42 2.35-5.88
	Type IV						
	M 94 F 20	48 \pm 1 55 \pm 3	4.20 \pm 0.28 3.40 \pm 0.59	274 \pm 5 308 \pm 15	1.08 \pm 0.01 1.08 \pm 0.03	7.78 \pm 0.09 3.86 \pm 0.36	1.51-4.97 1.37-6.45
	Type V						
	M 3 F	46 \pm 7 18	1.1 \pm 1.20 1.1 \pm 1.20	353 \pm 87 353 \pm 87	1.06 \pm 0.09 1.06 \pm 0.09	2.02 \pm 0.63 2.02 \pm 0.63	0.84-2.98 0.84-2.98

Table II Comparison between regression equations for male and female subjects with hyperlipoproteinaemia. The r-value and Student's t-value are given for linear regression and double logarithmic regression.

	Linear regression		Double logarithmic regression	
	Males	Females	Males	Females
r	0.29	0.46	0.53	0.55
t	3.73	4.83	7.74	6.19

was of a hyperbolic than a linear type (20, 22, 23, 24). This has been tested in the present material and the results are given in Table II. The comparison between linear and double logarithmic regression revealed that higher r -values and Student's t values were found for the logarithmic regression supporting the concept of a hyperbolic relation between k_2 and plasma TG concentration. In the following presentations the relationships between k_2 and TG have therefore been analysed in double logarithmic plots.

The data in Table I represent very wide ranges of k_2 values and serum TG concentrations. Fig. 1 demonstrates that the mean values of these different groups together formed a negative relation between k_2 and serum TG also when the extreme values obtained from males with a type V pattern or male and female controls were included. The groups indicated in Fig. 1 represent different and distinct lipoprotein types by definition but still formed a continuous relation. All female groups, however, seem to be found to the right of the corresponding male group. Furthermore it is also possible that male and female subjects with a type III pattern were found towards the right part of the relation curve. Although the mean value for k_2 was lower in subjects with elevated serum TG concentrations there was a considerable overlap of hyperlipoproteinaemic and control subjects. Several subjects with hypertriglyceridaemia had higher k_2 values than individual controls with low TG values. This finding might illustrate that the lipoprotein typing system according to the WHO classification is a static way to define hyperlipoproteinaemia and does not take into account more dynamic aspects of the lipoprotein metabolism. There is now accumulating evidence that hyperlipoproteinaemia is a much more heterogeneous condition than was previously thought. In a recent paper Lees (30) has underlined the importance that data on plasma concentration of lipids are combined with kinetic parameters of lipoprotein metabolism in order to enable an accurate definition of the phenotypes that reflect the inherited biochemical defect underlying the

primary hyperlipoproteinaemias. The findings of the population study demonstrated that although a significant correlation between k_2 and serum TG was found in a large population there was a considerable overlap of k_2 values between subjects with elevated serum TG concentrations and controls. These observations prompted the present series of investigations with the general aim to study whether the IVFTT k_2 value could provide additional information about the dynamic aspects of lipoprotein metabolism and thus be of value in a more integrated system to classify hyperlipoproteinaemia.

Boberg, Carlson and Hallberg suggested that the IVFTT might be of clinical value in several ways. As a method to study physiological and patho-physiological aspects of TG metabolism as a tool to evaluate the effects of treatment of hyperlipoproteinaemia and as a method that could possibly discover subjects prone to hypertriglyceridaemia and coronary heart disease.

If this would hold true the IVFTT would resemble the intravenous glucose tolerance test (IVGTT) in several aspects as a tool for metabolic studies. Both methods determine the elimination course of a substance given intravenously as a single dose injection.

When the IVFTT was introduced it was unknown to what extent the test could be applied in the examples mentioned above. Furthermore several methodological problems remained unsolved. In the first report on the IVFTT exogenous TG were first separated from endogenous TG by a PVP gradient method (31) followed by determination of the TG concentration in the isolated fractions. This method is a very tedious procedure and not suitable to apply if the IVFTT should serve as a clinical routine method.

AIM OF THE PRESENT STUDY

1. To develop a clinical routine method to perform the intravenous fat tolerance test (IVFTT) in man and experimental animals and to develop a laboratory method to analyse the disappearance of Intralipid[®] from the blood stream.

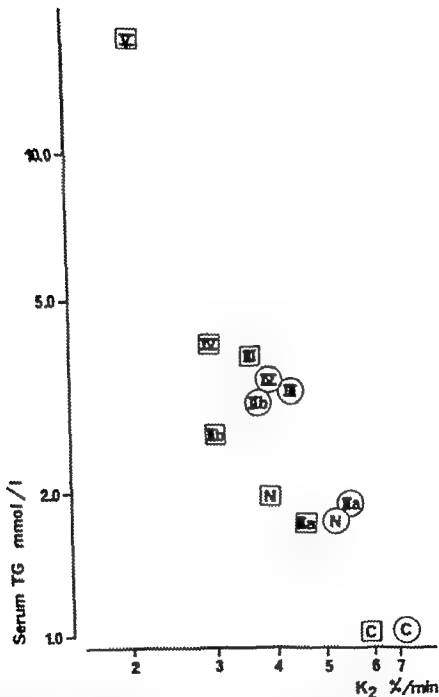


Fig 1 Relationship between k_2 and serum TG in groups of subjects with varying concentrations of lipoproteins (Table I) C = control subjects, II = subjects initially classified as hyperlipoproteinaemia but with sera typed as normal on subsequent analysis. Roman figures indicate type of hyperlipoproteinaemia. Males in open squares, females in open circles. Logarithmic scales.

2. To investigate whether the IVFTT could reflect fractional turnover rate also of endogenous plasma TG
3. To investigate in which tissues the Intralipid® emulsion is removed
4. To apply the IVFTT in the study of individuals whose plasma lipoprotein concentrations were changed either by disease or by treatment with drugs and to study the relationships between k_2 and lipoproteins after such manipulations in the same individuals

SUBJECTS

In the methodological study (I) male and female volunteers and patients with various types of hyperlipoproteinaemia were studied. In study II male subjects with "normal" or elevated plasma TG concentrations according to the Stockholm Prospective Study (32) were investigated. In studies III and V male volunteers with

previous history or symptoms suggestive of cardiovascular or metabolic disorder were studied. In study IV patients, admitted to the department of surgery, Karolinska Hospital for cholecystectomy were studied. These patients were all free of symptoms suggestive of acute cardiovascular or metabolic disease and were not on any medication expected to affect lipid metabolism. In all patients an informed consent to the study according to the Helsinki declaration was obtained. No complications were observed and the recovery after the operation was uneventful in all cases. In study VI 11 healthy female premenopausal subjects without any history or symptoms indicating a metabolic disorder were studied. Study VII comprised nine female hypothyroid patients.

METHODS

The intravenous fat tolerance test (II)

The IVFTT was always carried out after an overnight fast and with the subjects in supine position. An indwelling catheter was inserted into an antecubital vein and was used both for Intralipid® injection and blood sampling. The

following routine method for the intravenous fat tolerance test was developed. A single dose of the fat emulsion (1 ml 10 % Intralipid®/kg body weight) was given intravenously. Blood samples were drawn every five minutes for forty minutes and the light scattering index of plasma diluted 1:100 in saline was determined by means of the Thorp micronephelometer (33). Capillary samples could be used as well, which makes it possible to apply the method when there are difficulties to use a permanent intravenous cannula such as in patients who do not have veins that are easily accessible in children or in laboratory animals. In earlier studies on the IVFTT exogenous TG were separated from endogenous TG by a PVP-gradient technique (31). This method is tedious and so a method using the Thorp micronephelometer was developed. Comparisons between nephelometry and PVP-gradient determinations have previously been shown to agree excellently (34). Since the start of the present study more than 2100 IVFTTs have been carried out. No adverse reactions of any kind have been observed.

Comparisons between exogenous and endogenous plasma TG fractional turnover rates (II)

The fractional removal rate determined by the IVFTT was compared with the fractional turnover rate of endogenous TG in paper II where the plasma TG turnover had been quantified (35). In these studies the endogenous plasma TG turnover was studied in two ways based on either a chemical secretion method or a "plasma clearance method". The first method determines the splanchnic secretion rate of unlabelled plasma TG calculated as the concentration difference between artery and the hepatic vein multiplied by estimated hepatic plasma flow. The second method estimates the amount of TG removed from the circulation over a certain period of time by means of labelled TG. The value of the "fractional turnover rate" of endogenous plasma TG was calculated by dividing the value of plasma TG turnover rate by the plasma TG concentration.

Procedures in study III, IV and V

In three studies (III, IV, V) the removal of Intralipid® in the following tissues was studied. The myocardium (III), the splanchnic region and the liver (IV) and the skeletal muscles and subcutaneous tissue of the forearm (V). The procedures which were used in these studies are described in detail in the individual papers. In this section they are only briefly summarised.

All subjects were studied in the morning after an overnight fast. In study III the concentration differences of Intralipid® across the myocardium were determined by means of catheters in a brachial artery and in the coronary sinus. In study IV a brachial artery, the hepatic vein and the portal vein were catheterised during anaesthesia and the concentration differences of the fat emulsion were determined across the splanchnic region and across the liver. In study V a brachial artery, a deep and a superficial antebrachial vein were catheterised and concentration differences were studied across the forearm skeletal muscle and across the forearm subcutaneous tissue.

In these three studies blood samples were drawn simultaneously from the catheters used for sampling. Dry syringes were always used for the samples for nephelometry and blood was taken into tubes with hypodilised heparin. No heparin was used to keep catheters patent but only slow saline drops or intermittent flushes with saline.

Plasma water shifts

As it was to be expected that any existing concentration differences would be small, it was essential that any systematic shift of plasma water which might occur during the passage of the blood across the systems studied could be detected. To establish if such shifts of water did occur ^{125}I -albumin was used as a tracer for plasma albumin. It was given to the subjects studied in III, IV and V one to four days before the study. In replicates of samples drawn from the respective catheters the ^{125}I radioactivity was subsequently determined. With this method it should be possible to detect shifts in the order of 1% of the arterial concentration.

Clinical studies

In studies VI and VII the lipoprotein metabolism was studied in patients where the plasma lipoprotein concentration was increased. In study VI healthy female subjects without clinical symptoms of metabolic disease were studied before and during treatment with an estrogen containing oral contraceptive. In study VII by postthyroid female patients were studied before and during substitution with thyroid hormone. In both these studies plasma lipids, lipoproteins and the IVFTT were determined before and during drug treatment.

DISCUSSION OF METHODS

In studies III, IV and V the Intralipid® uptake in various tissues was studied by means of nephelometry determinations of exogenous TG. In these studies the assumption has been made that nephelometric concentration differences across a vascular bed indicate a removal of Intralipid® particles and that no change in particle size appears. This assumption may be valid for the following reason. In study III the nephelometric arterio-coronary sinus differences were compared with a chemical method where plasma TG was determined by measuring total plasma glyceride-glycerol by an AutoAnalyzer technique (77). These values were corrected for free glycerol (35). The grand means for the two methods in study III were for the chemical method $54 \pm 24 \mu\text{mol/l}$ nephelometric method $46 \pm 27 \mu\text{mol/l}$. In studies IV and V a comparison was made between the nephelometric determination of Intralipid® TG uptake and a chemical method where lipids were extracted by means of a chloroform-methanol Folch extract on which triplicates were analysed ten times each (7, 36). This chemical determination gives a very high precision. In the subjects studied, a close correlation was found between the nephelometric data and the values from the chemical method. The grand means for the two methods in studies IV + V were for the chemical method $66 \pm 18 \mu\text{mol/l}$ and for the nephelometric method $67 \pm 8 \mu\text{mol/l}$. These results taken together suggest that nephelometry is a

valid method to determine concentration differences of Intralipid[®] over the vascular beds studied in papers III, IV and V

RESULTS AND COMMENTS

Methodological studies on the IVFTT (I)

In study I it was shown that a linear standard curve was obtained when Intralipid[®] was diluted. This curve included the origin. This finding made it possible to use a standard curve for the conversion of plasma light scattering index (LSI) to μmol Intralipid[®] TG/l. In studies III, IV and V. When 1 ml 10% Intralipid[®] per kg body weight was given, first order kinetics were generally demonstrated for the removal of the fat emulsion. The slope of the regression line was determined by the method of the least squares and the equation $\log Y = bX + a$ was calculated. The disappearance rate k_2 of the fat emulsion was expressed as $-b/s_b$, where s_b is the standard error of b . A typical example of the data obtained from an IVFTT is given in Fig. 2. The slopes by which k_2 was determined were generally linear. No upward slope towards the end of the curve was found, which makes it unlikely that circulating "secondary particles" (37) have interfered with the nephelometric readings.

In study I it was demonstrated that the plasma samples could be stored at least two weeks before reading without affecting the k_2 value or its methodological error. This finding is of practical value in a laboratory routine. k_2 was highly reproducible in the same individual when repeated consecutive days. Further studies have demonstrated that a similar good reproducibility is found when subjects are retested with an interval from one week up to six months (Fig. 3) (38).

Comparison between IVFTT and endogenous plasma TG fractional turnover rates (II)

In 32 male subjects with wide ranges of the plasma TG concentration the fractional turnover rate of endogenous TG was determined and the IVFTT was carried out. A significant correlation between fractional turnover rate of

endogenous TG as determined by these methods and the IVFTT was obtained. The r -value for the relationship between the "chemical secretion method" and the IVFTT was 0.60 ($p < 0.01$) and the r -value for the relationship between the "clearance method" and IVFTT was 0.71 ($p < 0.001$). Because of methodological reasons the error for the determination of endogenous plasma TG turnover rate is quite high, around 40%. A statistical analysis of the relationships revealed that still higher r -values would have been observed if the methodological error of the endogenous plasma TG turnover study had been lower.

In this study a significant negative correlation was found between the fractional removal rates of both endogenous and exogenous plasma TG and VLDL-TG ($r = -.65 - -.078$).

Intralipid[®] removal sites (III, IV, V)

In studies III, IV and V the arterio-venous concentration differences of Intralipid[®] TG particles were studied by means of nephelometry across various tissues. In study III it was demonstrated that about 6% of the arterial concentration of Intralipid[®] TG was removed during the passage through the myocardial tissue. In study IV the arterio-portal concentration difference of Intralipid[®] averaged about 5% of the arterial concentration. There was however no significant portal-hepatic venous concentration difference of Intralipid[®] TG across the liver. In study V the corresponding figures for removal of Intralipid[®] TG particles were about 10% in the forearm skeletal muscles and 6% in the forearm subcutaneous tissues.

Blood plasma flow during the Intralipid[®] infusion was estimated in study IV. This study demonstrated that the Intralipid[®] infusion did not change the estimated splanchnic plasma flow. Under the assumption that the plasma flow was unchanged in the other tissues during a constant infusion of Intralipid[®]. It is possible to make a rough estimate of the role of the various tissues in the removal of Intralipid[®] from the circulation (Table III). The figures for plasma flow have been taken from Wade *et al* (39) and the hematocrit has been assumed to

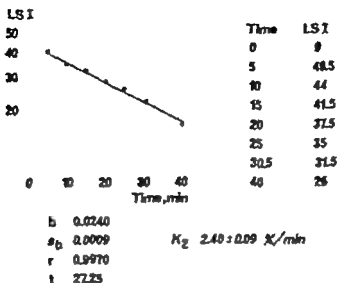


Fig. 2. Disappearance curve of Intralipid[®] during an intravenous fat tolerance test. After subtraction of a blank taken at zero time the light scattering indices are plotted against time in a semilogarithmic plot. By means of a standard computer program the slope of the curve is determined by the method of least squares. The disappearance rate k_2 of the emulsion is expressed as $-b/s_b$ and given as the percentage of the emulsion cleared from plasma per minute. The r -value and Student's t are also given.

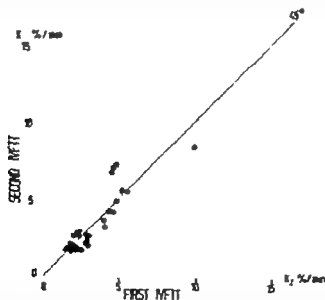


Fig. 3. Relationship between k_2 values calculated from IVFTTs carried out with time intervals between one week and six months. The individuals studied were not on medications or diets expected to affect the k_2 values or serum TG concentrations and had a wide range of fasting serum TG concentrations. The IVFTT was carried out in identical ways on both study occasions. The line of identity is indicated in the plot.

average 40%. In the calculation presented in Table III it has further been assumed that the subjects studied are resting supine as muscular exercise possibly can influence the removal of Intralipid® TG particles (Study V). In study IV the patients studied were anaesthetised whereas in studies III and V they were conscious and not premedicated. The possible Intralipid® removal differences induced by the anaesthesia given to patients in study IV have therefore not been taken into account in the calculation either. Under these assumptions and bearing in mind that the data in Table III are combined from different studies and calculated in each study separately it seems possible to account for the removal of Intralipid® in various tissues. Table III demonstrates that almost 50% of the infused Intralipid® was removed in skeletal muscle whereas the splanchnic viscera (except the liver) removed 25% of the infused Intralipid®. The figures for the per cent removal in the myocardium and subcutaneous tissues were about the same 13% in both tissues. The sum obtained from the different studies (99%) is as close to 100% as can be expected with regard to the methodological errors involved in determination of small arteriovenous concentration differences as well as all assumptions.

In hypertriglyceridaemia a low k_2 is a common finding as can be seen from data in Table I and from several clinical studies. The k_2 value is determined by means of an intravenous dose of Intralipid® and the elimination of Intralipid® is followed for approximately the same period of time during which the Intralipid® removal was determined in studies IV and V. It is therefore of great interest to analyse the data from Table III. According to these data the skeletal muscle seems to play a very important role in the removal of Intralipid® from the circulation. It is possible that an impaired removal process in skeletal muscle may at least partly account for the low k_2 values observed in hypertriglyceridaemia. Although the present studies do not provide information about the fate of the Intralipid® fat particles removed in various tissues it

is tempting to speculate about the possible importance of skeletal muscle in the regulation of the plasma TG pool size and as a possible "critical" tissue for the development of hypertriglyceridaemia.

Clinical studies on the IVFTT (VI-VII)

The relations between the IVFTT k_2 value and serum TG and lipoproteins were analysed in two clinical studies. In study VI healthy female subjects were treated with an estrogen containing oral contraceptive for two months. During this period of time the mean plasma TG concentration rose from 0.92 ± 0.09 mmol/l (SEM) to 1.70 ± 0.11 mmol/l ($p < 0.001$). This increase was due to statistically significant increases of TG in all three lipoprotein classes. Although the medication was withdrawn after two months, the TG concentration remained significantly elevated for four weeks. The IVFTT k_2 value remained unchanged by the treatment. The hypertriglyceridaemia thus seemed to be due to an increase turnover of plasma TG probably by a raised hepatic secretion. The role of the IVFTT as an additional tool in elucidating changes of plasma TG turnover will be discussed below.

In study VII hypothyroid patients were studied. It was demonstrated that in the hypothyroid state hypertriglyceridaemia can be found concomitant with the classical laboratory features of hypercholesterolaemia. Hypertriglyceridaemia generally is due to an increase of VLDL-TG but study VII seems to be the first demonstration of hypertriglyceridaemia due mainly to an LDL-TG concentration increase. It was shown that VLDL-TG was not affected by substitution whereas the LDL-TG concentration values were considerably reduced. When the hypothyroid subjects were compared to a randomly selected material of healthy female subjects of corresponding age it was further demonstrated that hypothyroid patients had a serum lipoprotein pattern which resembled a type III lipoprotein pattern since the cholesterol/TG ratio in VLDL was increased whereas

Table III. Intralipid® removal tissues. Estimated plasma flow fractional removal rate and per cent removal of infused Intralipid® in these tissues. The data were obtained from four different studies and each study was calculated separately

	Estimated plasma flow ml/min	Fractional removal rate % of arterial concentration	Removal of infused Intralipid® %
Myocardium	180	6	14
Splanchnic viscera	(Determined individually in study IV)	5	25
Skeletal muscle	720	10	47
Subcutaneous tissue	300	6	13
			99

"floating β " could not be demonstrated. After substitution the cholesterol/TG ratio in VLDL was normalised. Hypothyroid subjects had significantly lower k_2 values before than after substitution. When k_2 was plotted against VLDL-TG in patients with hypothyroidism a comparison with hypertriglyceridaemic control subjects revealed that hypothyroid patients seemed to have a reduced plasma TG turnover in spite of their serum TG concentration increase.

k_2 and plasma TG fractional turnover rates

The significant hyperbolic correlation between k_2 and plasma TG concentration could be explained either if the high plasma TG had been induced by defect removal mechanisms or if the injected Intralipid® competed with endogenous plasma TG about the removal processes. According to the former explanation the low k_2 obtained in hypertriglyceridaemia would reflect an existing impairment of TG removal processes while the latter concept indicates that the low k_2 in hypertriglyceridaemia was caused by the hypertriglyceridaemia in itself without reflecting the existing fractional turnover rate. According to this concept an increase of the plasma TG pool size will result in a decrease of k_2 and vice versa. There is however evidence that the fractional removal rate k_2 is a process that

may determine the TG pool size. Hallberg has demonstrated that the fractional removal rate is not affected by the constant infusion of Intralipid® with various infusion rates (40) in a study by Chaff & al (41) patients with alcoholic lipaemia were studied with the IVFTT on admission to the hospital and reinvestigated when their lipid levels were normalised after treatment with diet. Although TG was markedly reduced after alcohol withdrawal k_2 did not rise. In study VI k_2 was carried out in healthy female subjects before and during treatment with an oral contraceptive containing an estrogen derivative. The TG concentration rose significantly but k_2 remained unchanged. In a study by Gustafson and Sannerstedt (23) k_2 increased significantly after treatment with a lipid lowering drug although the plasma TG concentration was not significantly changed. In a recent study by Nestel the plasma TG concentration was manipulated by various diets (42) and the clearance rate of Intralipid® was studied. Although the plasma TG concentration was doubled after five days of an 80% carbohydrate diet the Intralipid® clearance remained unchanged. From these studies it seems reasonable to suggest that k_2 is not merely a reflection of the plasma TG pool size. This suggestion is further strengthened by the finding in study II where in fact a significant positive relationship between the k_2

log TG

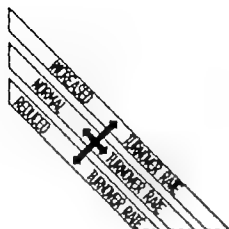
log k_2

Fig. 4 Model for the relationship between k_2 and plasma TG at different turnover rates. The hyperbolic relation between k_2 and TG corresponds to a straight line in a double logarithmic system. When k_2 and TG vary in such a system they vary inversely and thus the turnover rate is constant as long as the product line of these variables is found along the same turnover rate line

value and the fractional turnover rate of endogenous TG was demonstrated

The relationship between k_2 and the plasma TG pool size can be analysed by means of Fig. 4. Such an analysis of the relationships between k_2 and total plasma TG or VLDL-TG cannot yield information about the amounts of TG turned over per time unit. However such an analysis may assist in understanding the mechanisms that may operate in the regulation of the plasma TG pool size. Under the assumption that k_2 reflects endogenous plasma TG fractional removal rate (Study II) changes of k_2 and plasma TG concentration can in principle lead to three different turnover results.

- 1) If k_2 is increased concomitant with a fall of the plasma TG concentration a shift will be observed along the "normal turnover" line. The multiplication of total plasma TG by k_2 is a very crude estimate of plasma TG turnover and certainly does not take into account heterogeneity of the various TG containing

lipoprotein fractions. Furthermore the k_2 values were higher than the corresponding values for fractional turnover rate of VLDL-TG in study II. However bearing these limitations in mind the change would indicate that the improved peripheral elimination of TG results in a reduction of the plasma TG concentration. The turnover of plasma TG is however not changed. These changes of k_2 and plasma TG have been observed in previous studies on the effects of lipid lowering drugs such as nicotinic acid, oxandrolone and clofibrate derivatives (21-25, 43). In Fig. 5 this pattern is demonstrated with data from ref. 21.

- 2) If the k_2 remains constant but the TG concentration is increased, other mechanisms than a reduction of the peripheral elimination rate at least partly lead to the observed increase of the plasma TG pool size. Such a mechanism is described in the study of the effects of an oral contraceptive containing estrogen (Study VI) and is summarized in

Fig. 6 This mechanism has also been demonstrated in the study by Chait & al on patients with alcoholic lipaemia (41) If the product of k_2 and the plasma TG pool size is used as a measure of the turnover an increase is found. It is possible that stimulation of hepatic secretion of plasma TG into the circulation causes this effect (44).

3) If k_2 is reduced and the plasma TG pool size

is normal or slightly elevated, the $k_2 \times \text{TG}$ product will be low. In study VII VLDL-TG and k_2 in hypothyroid patients were slightly lower than observed for a group of hyperlipoproteinaemic females. Substitution with thyroid hormone changed these data towards normalisation. In hypothyroid subjects it is thus possible that the turnover of VLDL-TG is low.

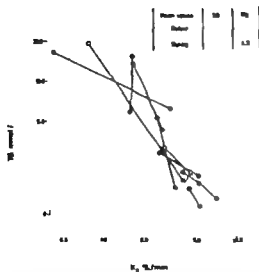


Fig. 5 Relationship between k_2 and plasma TG in ten male patients with hyperlipoproteinaemia before and during treatment with 4.5 g nicotinic acid/day. Mean values of k_2 and TG before and during treatment are given. Logarithmic scales.

Mean \pm SEM	k_2	TG
○ Before	8.1 \pm 0.9	0.9 \pm 0.1
● During	8.7 \pm 1.0	1.7 \pm 0.1

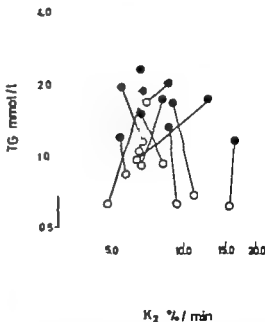


Fig. 6 Relationship between k_2 and plasma TG in twelve healthy female subjects before and during treatment with an oral contraceptive agent containing an estrogen derivative. Mean values of k_2 and TG before and during treatment are given. Logarithmic scales.

GENERAL SUMMARY

- 1 A routine method for the intravenous fat tolerance test (IVFTT) was developed using the fat emulsion Intralipid®
- 2 When 1 ml 10% Intralipid®/kg body weight was given, first order kinetics for the removal of the fat emulsion were generally found and linear curves were obtained in a semi-logarithmic system.
- 3 The IVFTT k_2 value (fractional turnover rate of Intralipid®) was highly reproducible when repeated in the same subjects with intervals of up to six months
- 4 There was a statistically significant positive relationship between the k_2 value and the fractional turnover rate of endogenous triglycerides (TG). Furthermore there was a significant negative relationship between k_2 and very low density lipoprotein TG
- 5 In subjects with various types of hyperlipoproteinemia and with wide ranges of k_2 values and serum TG concentrations, statistically significant negative relationships between k_2 and TG were observed. The statistical significance of these relationships was considerably improved when the relations were studied in double logarithmic systems.
- 6 The arterio-venous concentration differences of Intralipid® during a constant infusion

were studied in several tissues and the following results were obtained. Intralipid® was significantly removed in the myocardium, the splanchnic region, the forearm skeletal muscle and subcutaneous tissue. No net removal was observed in the liver. If the figures obtained from these studies were extrapolated to whole body removal of Intralipid® it was possible to account for 99% of the total Intralipid® removal.

- 7 The Intralipid® removal in skeletal muscle was studied for the same period of time during which the Intralipid® removal was followed by the IVFTT. During this period of time almost 50% of the Intralipid® was removed in the skeletal muscle. This major role of skeletal muscle suggests that the negative relationship between k_2 and serum TG concentrations may be due to a decreased TG clearance by skeletal muscle in hypertriglyceridaemia.
- 8 In hypothyroid females hypertriglyceridaemia due to an increase mainly in low density lipoprotein TG was demonstrated.
- 9 From the findings in the present series of studies it was suggested that the IVFTT k_2 value is not a reflection of the plasma TG pool size but rather a process at least partly involved in the determination of the plasma TG concentration.

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REFERENCES

- 1 Carlson L. A. Serum lipids in men with myocardial infarction *Acta med scand.* 167:339 1960
- 2 Albrink M. J., Meigs J. W. and Man E. B. Serum lipids, hypertension and coronary artery disease *J Med* 31:4 1961
- 3 Carlson L. A. and Böttiger L. E. Ischemic heart disease in relation to fasting values of plasma triglycerides and cholesterol. *Lancet* ii:865 1972
- 4 Fredrickson D. S., Levy R. I. and Lees R. S. Fat transport in lipoproteins. An integrated approach to mechanisms and disorders. *N Engl. J Med* 273:32 1967
- 5 De Lalla D. E. and Gofman, J. W. Ultra centrifugal analysis of serum lipoproteins *Meth. biochem Anal* 1:459 1954
- 6 Lees, R. S. and Hatch F. T. Sharper separation of lipoprotein species by paper electrophoresis in albumin-containing buffer *J Lab Clin Med* 61:518 1963
- 7 Stone M. C. and Thorp J. M. A new technique for the investigation of the low density lipoproteins in health and disease *Clin. chim. Acta* 14:812 1966
- 8 Carlson Kerstin. Lipoprotein fractionation. *J Clin. Path.* 26 suppl (Ass Clin. Path.) 5:32 1973
- 9 Robinson D. S. The function of the plasma triglycerides in fatty acid transport. In *Lipid metabolism* (eds M. Florkin and E. H. Stoltz) p. 51 Elsevier Publishing Company Amsterdam 1970
- 10 Boberg J. Carlson L. A., Freyschuss, Ulla, Lassers B. W. and Wahlqvist M. L. Splanchnic secretion rates of plasma triglycerides and total and splanchnic turnover of plasma free fatty acids in men with normo- and hypertriglyceridaemia *Europ J Clin Invest* 2:454 1972
- 11 Angerwall G. On the oral fat tolerance test *Acta med scand* 176 suppl. 424 1964
- 12 Carlson L. A. and Hallberg, D. Studies on the elimination of exogenous lipids from the blood stream. The kinetics of the elimination of a fat emulsion and of chylomicrons in the dog after single injection *Acta physiol. scand* 59:52 1963
- 13 Hallberg D. Studies on the elimination of exogenous lipids from the blood stream. The kinetics for the elimination of chylomicrons studied by single intravenous injection to man. *Acta physiol. scand* 65:279 1965
- 14 Morris, B. and French, J. E. The uptake and metabolism of ^{14}C -labelled chylomicron fat by the isolated perfused liver of the rat *Quart J exp Physiol* 43:180 1958
- 15 Olivecrona, T. and Belfrage P. Mechanisms for removal of chyle triglyceride from the circulating blood as studied with (^{14}C) glycerol and (^3H) palmitic acid-labeled chyle *Biochem Biophys. Acta* 98:81 1965
- 16 Meng H. C. Senaki H., Thompson S. W. Jones L. D. & al. Toxicity testing of fat emulsions I - IV *Am J Clin. Nutr* 16:29 37 43 62 1965
- 17 Schuberth, O. Clinical results of intravenous infusions of fat emulsions. *Nutr Dieta* 5:387 1963
- 18 Boberg J. and Carlson L. A. Determination of heparin-induced lipoprotein lipase activity in human plasma. *Clin. chim. Acta* 10:420 1964
- 19 Hallberg, D. Studies on the elimination of exogenous lipids from the blood stream. The kinetics of the elimination of a fat emulsion studied by single injection technique in man. *Acta physiol. scand.* 64:306 1965
- 20 Boberg J. Carlson L. A. and Hallberg, D. Application of a new intravenous fat tolerance test in the study of hypertriglyceridaemia in man. *J Atheroscl. Res.* 11:159 1969
- 21 Boberg J. Carlson L. A. Froberg, S.,

- Olsson, A., Orö L. and Rössner S. Effects of chronic treatment with nicotinic acid on intravenous fat tolerance and postheparin lipoprotein lipase activity in man. In *Metabolic effects of nicotinic acids and its derivatives* (Eds. K. F. Gey & L. A. Carlson) p. 465 Hans Huber Publishers, Bern, Stuttgart Vienna 1971
- 22 Lewis, B. Mancini, M., Mattock, M., Chait A. and Russel Fraser T.. Plasma triglyceride and fatty acid metabolism in diabetes mellitus. *Europ J Clin. Invest.* 2:445 1972.
- 23 Gustafson, A. and Sannerstedt R.. Drug treatment of lipoproteinaemia Type II Effect on plasma lipids of a new phenoxycetic derivative clofibrate and nicotinic acid *Europ J Pharmacol* 5:259 1973
- 24 Nückl, E. and Kekki, M. Plasma triglyceride transport kinetics in diabetes mellitus. *Metabolism* 22:1 1973
- 25 Olsson, A. G. Orö L. and Rössner S.. Effects of coarandolone on plasma lipoproteins and the intravenous fat tolerance in man *Atherosclerosis* 1974 in press.
- 26 Rössner S., Olsson, A.G. and Carlson L. A. The intravenous fat tolerance test in normo- and hyperlipoproteinaemic subjects. In preparation
- 27 Kessler G. and Lederer H. Fluorometric measurement of triglycerides. In *Automation in analytical chemistry* (Ed. L. T. Skeggs) vol. 1 p. 341 Mediad Inc. New York 1965
- 28 Block, W. II. Jarrett K. J. and Leone B. Use of a single color reagent to improve the automated determination of serum total cholesterol. In *Automation in analytical chemistry* (Ed. L. T. Skeggs) vol. 1 p. 345 Mediad Inc. New York 1965
- 29 Beaumont J.L., Carlson, L. A., Cooper G. R. Fejfar Z., Fredrickson D. S. and Strasser T. Classification of hyperlipidaemias and hyperlipoproteinaemias. *Bull. Wld Hlth Org* 43:691 1970
- 30 Lees, R. S.. A progress report on lipoprotein phenotyping. *J Lab Clin Med.* 82:529 1973
- 31 Gordis, E. Demonstration of two kinds of fat particles in alimentary lipaemia with polyvinylpyrrolidone gradient columns. *Proc. Soc. exp Biol. Med* 110:657 1962
- 32 Carlson L. A. and Lindstedt S.. The Stockholm Prospective Study I *Acta med. scand suppl.* 493 Stockholm 1968
- 33 Thorp J M., Hornfall G. B. and Stone C. II Technical note A new red-sensitive micro-nephelometer *Med. Biol. Engng* 5:51 1967
- 34 Lewis, B., Boberg, J., Mancini M. and Carlson L. A. Determination of the intravenous fat tolerance test with Intralipid® by nephelometry *Atherosclerosis* 15:83 1972
- 35 Boberg J. Carlson, L. A. and Freyschuss, Ulla. Determination of splanchnic secretion rate of plasma triglycerides and of plasma free fatty acid total and splanchnic turnover *Europ J Clin. Invest* 2:123 1972.
- 36 Carlson L. A.. Determination of serum triglycerides. *J Atheroscl. Res.* 3:334 1963
- 37 Dole V. P. and Hamlin, J. T.. Particulate fat in lymph and blood *Physiol. Rev* 42:574 1962
- 38 Rössner S.. Studies on the reproducibility of the intravenous fat tolerance test. In preparation.
- 39 Wada O. L., Bishop J. M. and Donald K. W. Cardiac output and regional blood flow Blackwell Scientific Publisher Oxford 1962
- 40 Hallberg D.. Studies on the elimination of exogenous lipids from the blood stream. The kinetics of the elimination of a fat emulsion studied by a constant infusion technique in man. *Acta physiol. scand* 64:299 1965
- 41 Chait A. Mancini, M., February A. W. and Lewis, B.. Plasma triglyceride metabolism

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Intravenous Glucose Tolerance and Early Insulin Response

Studies on a random sample of women aged 50 and in patients with diabetes mellitus

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By Göran Blohme

in collaboration with
Ragnhild Arridsson Lenner
Johan Waldenström

Göteborg 1974

To Brother
Christina
and Magnus

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INTRODUCTION

The international consensus of opinion that the prevalence of clinically manifest diabetes mellitus is increasing in most parts of the world (51). A number of factors have been blamed for this increase including improved standard of living and altered genetic composition of the population.

Screening campaigns for diabetes and more frequently occurring regular health controls have undoubtedly revealed an increased number of previously unknown cases of mild diabetes especially among the elderly. About 1/3 of the total number of diabetic cases considered to be unknown at given point of time (52).

The prevalence of manifest diabetes is strongly dependent especially in women (52-72). It has been calculated that about 10 percent of women and 5 percent of men in Sweden will develop manifest diabetes during their lifetime (72). Still more subjects would have been made diabetic if they had not died from other causes.

The high total morbidity for diabetes and the well known relationship of the disease as well as the association between diabetes and the atherosclerotic vascular disease (63-129-154-166) indicate that diabetes constitutes a major health problem now in many countries. Although the need for further study and prevention is

The manifest form of the disease is considered to present only a very small part of the diabetic syndrome which also includes minor degrees of impairment of the glucose homeostasis as well as the so-called prediabetic state (51). Large screening investigations have indicated that an impaired glucose tolerance is not uncommon in the general population especially among the elderly (25-52-83). While generally impaired glucose tolerance is generally accepted to indicate a definite threshold of border line tolerance for the future development of manifest diabetes quantitative data by long term follow-up studies (29-131-133).

In clinical studies have been widely used in the investigation of the pathogenesis of the diabetic syndrome during recent years (105-138). It has been focused on the clinical phases of the condition in studies on subjects with minor degrees of disturbance in the glucose

homozygous or a genetic predisposition for diabetes owing to the hypothesis that all stages of the diabetic syndrome including the prediabetic state might be characterized by a low and delayed early response (35). Few data are available concerning the distribution of the magnitude of the early insulin response and the evaluation of subnormal responses in the general population. Conditions for population studies are good in Sweden as a representative and up to date sample of the population can easily be obtained from the Revenue Office Register. Experiments in previous population studies in Göteborg, Sweden, indicate that a high participation rate may be anticipated (77, 163).

The present cross-sectional study of women aged 50, part of a larger population study of middle-aged women randomly selected from the general population in Göteborg, Sweden. A large number of such projects have been performed in one way or another by Bengtsson et al. (14). Results have in part been presented elsewhere by Bengtsson et al. (15) and Hallstrom (78).

The present investigation of randomly selected women was supplemented by a study of middle-aged patients with maturity-onset diabetes selected from the same area of the population sample.

The main purpose of the present investigation in women aged 50 was

1. to study the glycosylated haemoglobin as an indicator of average glucose level and to turn to the prevalence of impaired tolerance
2. to study the daily glucose turnover rate inulin, possibly affected by insulin resistance inulin concentration in the peripheral blood and to estimate the prevalence of a low response in non-patient with manifest diabetes and in women with impaired glycosylated haemoglobin
3. to study the correlation between the individual family history of diabetes and the other glycosylated haemoglobin and daily inulin response
4. to study whether dietary habit and body composition might influence glycosylated haemoglobin and daily inulin response
5. to obtain a basis for prospective study with the principal aim of assessing the genetic influence on maturity-onset diabetes for the future development of manifest diabetes

MATERIAL AND METHODS

Material

The present investigation was performed in Göteborg, Göteborg the county in Sweden. It is situated on the west coast and had about 445 000 inhabitants in 1968. Further details concerning Göteborg have been presented elsewhere (60, 163).

The population sample

A random sample of women in the age groups 38, 46, 50, 54 and 60 was obtained from the Revenue Office Register (14). The sample consisted of women born in Sweden which was a simple random sample of the population (6, 12, 18, 24, 30). Women aged 50 (born 1918) in total 436 were selected for the present investigation. A participation rate of 91 percent was obtained in the preliminary examination (14). A mail subsample (n = 20) of women aged 50 had been taken part in a pilot study before the main population study started in autumn 1968. This subsample was included in the present investigation concerning intravenous glucose tolerance and plasma insulin response. The total sample for this part of the study consequently consisted of 416 women (Table I). A total of 352 women without manifest diabetes were studied which corresponded to a participation rate of 85 percent and 87 percent of women available for study.

Table I The population sample of women aged 50

	n	%
Participants	352	84.6
Non-participants	64	15.4
Died in illness	5	1.2
Uremia	1	0.2
Died	3	0.7
Moved from town	4	1.0
Refused to participate	51	12.3

Women aged 50 born on the 6th day of November months were selected for the investigation of dietary habits together with women with impaired glucose tolerance and/or subnormal insulin response and found in chapter VII. This will be presented in more detail in that chapter.

The women in this population sample had been extensively studied at the primary examination (14). All women taking part in this present investigation had normal serum creatinine level. The questionnaire and the physical examination did not reveal current liver disease in any of the women. No liver function tests were performed in this age group. The prevalence of angina pectoris was 3 per cent and coronary ECG type was normal (13). Eleven per cent of the women participating in the intravenous glucose tolerance test (IVGTT) had a blood glucose exceeding 160/95 in the state depicted at the primary examination (13) measured as recommended by WHO (7). About 5 per cent of the women were treated with antidiabetic drugs owing to hypertension during the period before the IVGTT.

Serum lipids were analysed in all women. Detailed report on serum cholesterol and serum triglyceride concentration have been presented elsewhere (13, 15). The serum cholesterol concentration ranged from 154 to 492.5 mg/100 ml with a mean of 276 mg/100 ml and a value of the 9th decile of 335 mg/100 ml. The serum triglyceride concentration ranged from 0.42 to 4.94 mmol/l with a mean of 1.26 mmol/l and a value of the 9th decile of 1.85 mmol/l.

None of the women was taking oral contraceptives.

Some further data on the material is presented in table II.

Non-participants: A few of the sample of 416 women had died or had moved away from the town during the interval between the sampling and the intended examination date (Table I). A mailing up of women (31) failed to participate in the first examination. Five women with manifest diabetes were found at the first examination and one woman with a normal fasting blood sugar was found. Another 20 women were found at the primary examination and did not go to participate in the subsequent IVGTT. The mean relative body weight of the women who failed to participate in the primary examination and/or the IVGTT (n = 51) was 105.0 ± 20.4 per cent (mean \pm SD) as compared with 108.7 ± 16.5 per cent (mean \pm SD) for the women who participated.

T b l l l D t o n p t r p a n t a n d f s t t h I V G T T M D m e i n g d a t a

		n	U n m	i d M	e d	D i v o	e d	W i d w	X ²	p
M t a l	P t i l p a n t	352	8 0	77 8	9 4	4 8				
t a t	R f	51	19 6	54 9	13 7	11 9			14 1	< 0 005
		N u m b e r f b i l d r e n								
P i t y	P t i l p a n t	352	0	1	2	3	≥ 4			
(%)	R e f	51	18 8	21 3	35 2	16 5	8 2		5 3	> 0 1
L a s b a b i	P t i l p a n t	286	I I b y ≥ 4 5 k g							
(% f w o m =	R e f	34	5 9						0 7	> 0 1
w i t h c h i l d n)										
F a m i l y	P a t p a n t	352	M t h f t h r b l i n g				O t h	I t		
b i t o y o f			19 6				12 8			
d i b i t	R f	41	10 4				8 3		3 7	> 0 1
(%)										

* M D I I
M D 3

in the IVGTT ($t = 1.4$, $p > 0.1$). Single women were over-represented among the failures (Table II). No significant differences were noted, however, in number of children, frequency of large babies (≥ 4.5 kg) or frequency of family history of diabetes between participating women and failures. The participation rate in the study of dietary habits will be discussed in chapter VII.

Death certificates were obtained from the Central Bureau of Statistics, Stockholm, for all women born in 1918 who had died in 1950 or later, and who by use of the sampling method would have been invited to the examination if still alive. Fifteen women had died during the period 1950-1967. The diagnoses of these women are presented in Table III. The diagnosis was based on autopsy findings in 73 per cent of the women.

Table III. Cause of death of 15 women who died between 1950 and 1967 and who by use of the sampling method would have been invited to take part in the population study.

Cause of death	n		n
Heart disease		Cancer	4
Thrombosed aorta	1	Hydramnios	2
Alcohol poisoning	1	Stroke	1
Pneumonia		Adenitis	1
Unknown	1		

By the method of selection the population sample is representative of the general population of women aged 50. In this population sample and completion of information by the participating women ensure that the results obtained are representative of the same population.

II The material of diabetic patients

Patients in the of an outpatient clinic followed by us entered into this study if they fulfilled the following criteria.

- 1 They were aged 40-62 (born 1910-1932)
- 2 They were referred to the clinic because of glycosuria without clinical symptoms of diabetes
- 3 They had on an empty stomach a fasting plasma blood glucose concentration exceeding 120 mg/100 ml
- 4 They had never been treated with insulin
- 5 They had not been treated with other medicinal therapy for diabetes during the last 12 months
- 6 They were otherwise in good health and with a stable body weight

According to the criteria 34 patients with mild or moderate maturity-onset diabetes were found, all of whom participated in the present investigation. Seven patients had hypertension and were treated with a β -blocker agent (n = 5), methylprednisolone (n = 1). One patient had previously suffered myocardial infarction and one had suffered from a heart attack. All had a normal hemoglobin level.

Comment: Middle-aged patients with diabetes were selected for the present study, although the influence of age on insulin secretion is reviewed by Johansson (96). The selected patients may be regarded as partly representing very mild forms of maturity-onset diabetes. The material will be discussed in detail in chapter VIII.

Method

Definition

Manifest diabetes mellitus in women is defined for the present population study as a condition in which the woman before the study had been previously treated with and/or medicinal therapy by physician and at the study had a fasting non-fasting plasma glucose concentration exceeding 120 mg/100 ml. One woman with previously unknown diabetes with a fasting blood glucose concentration exceeding 200 mg/100 ml was repeated analyzed and was found to have manifest diabetes. All the

was considered to have or have had manifest diabetes if prescribed diet and/or medicinal therapy by a physician.

A close family history of diabetes was considered present in women with a diabetic mother, father or sibling. Women with a diabetic grandparent, aunt, uncle, etc. on either half, both (n = 2) or own child (n = 2) were grouped together as other relatives. In the analysis in chapters II and VI in women who did not know about their relative family history was classified as negative.

Primary population study

The examination outline at the primary population study has been presented previously (14). Information concerning medical status was obtained from the Rönneby Office Registry. Information on number of children, age, babies and family history of diabetes was obtained from questionnaires and checked by interview in women participating in the IVGTT. Information concerning the patients' ages and if dead or deceased was obtained from questionnaires. The age of living parents was noted at the time of study. If a parent was deceased the age at death was noted.

The intervention: glucose tolerance test

The women who were subjected to the IVGTT were all ambulatory and came to the laboratory in the morning after fasting 12 hours. A diet with a carbohydrate content of about 300 g was prescribed during 3 days before the test. The women received a written food schedule specifying the amount of carbohydrate to be consumed at the various meals. The importance of the carbohydrate feeding was underlined. The attendance of the women started at the IVGTT, the day had followed the diet schedule. Drug was withdrawn for at least one day and smoking was not allowed during the last 12 hours before the test. An insulin syringe was assigned for the tests and the women were kept in the ambulatory position throughout the test. An indwelling needle was placed in an antecubital vein in the right arm and kept patent by low flow infusion. After an equilibration time of 15 minutes glucose 0.5 g/kg body weight was intravenously injected. Glucose 50 g was per 100 ml was injected intravenously during 5-30 minutes. Immediately before and at 14, 6 and 8 minutes after the start of the glucose injection blood samples were taken in the opposite arm for uric

in ulin and whole blood glucose determination Between 25 and 60 minutes venous blood samples were taken every 5 minutes for blood glucose determination

Blood glucose concentration was determined in duplicate in whole blood by means of a glucose oxidase method using a commercially available reagent (Glox[®] AB Kabi Stockholm) and with glycine buffer and potassium periodate as precipitating agent (111). The methodological error ($\sqrt{\frac{d^2}{2N}}$) difference between duplicate numbers of samples was 1.6 mg/100 ml. The coefficient of variation ($\frac{S.D. \times 100}{\text{mean}}$) was 2.0 percent at the 225 mg/100 ml level calculated from a fasting standard curve. The blood sampling and the termination of blood glucose concentration was in all instances aided out by a specially designed assistant.

Fasting venous blood glucose concentration was determined in women at the primary examination of the population study by means of a ferricyanide reduction method (88) adapted for autoanalysis by Thénin on N 26. The methodological error was 3.0 mg/100 ml. Capillary blood glucose concentration was determined in the diabetic patients (chapter VIII) at the outpatient clinic in the period before and after the IVGTT by means of an o-Toluidine method (81). The methodological error was 4.5/100 ml.

The mean in blood glucose concentration of fasting level at 4 and 6 minutes after the start of the glucose injection was used to calculate:

- the mean of increments at 4 and 6 minutes (G_{in})
- the mean of increments per minute during the first five minutes (G_{turn})

When calculating the G_{turn} it was assumed that the blood glucose concentration increased linearly from 0 to 4 minutes (162). The 5 minute concentration was obtained as the mean of 4 and 6 minute concentration.

The glucose tolerance was expressed as a k value representing the disappearance of blood glucose in percent per minute. The k value was obtained from the slope of total blood glucose on a logarithmic scale of venous blood glucose concentration between 25 and 60 minutes and finally described by Hamilton and Stein (79). The best

fit of the straight line was determined using the method of least squares. Blood glucose concentration 10 mg/100 ml or less above the fasting level were excluded because the blood glucose had then reached its steady state. An exception to this rule for calculation of the k value was made in four women with extremely high glucose disappearance rates. The k value had to be calculated from data points even below fasting level + 10 mg/100 ml (k values 4.96, 6.70, 6.72 and 9.86 per cent/h). The valuation of the k value together with results will be discussed in detail in chapter III.

Summation insulin concentration was determined by a double antibody method originally described by Hall and Randall (74) using a commercial radioimmunoassay kit (The Radiochemical Centre, Amersham, U.K.). The standard curve was constructed using human insulin dissolved with the kit. Inulin was determined in duplicate in the population sample and in triplicate in the diabetic patient. The methodological error at the 20 mU/l level calculated from duplicate summation was 1.4 mU/l in the population study and 0.9 mU/l in the study of diabetic patients. At the 50 mU/l level the corresponding values were 3 and 2 mU/l respectively. The coefficient of variation was found to be 11 per cent at the 17 mU/l level.

The plasma insulin response (ER) was calculated according to Thierlich (162) using the formula

$$\frac{df/dt}{f(t)} = kI$$

where

I = insulin concentration (mU/l)
 $f(t)$ = rate at (mU/l/min)
 k = elimination constant (fraction of plasma pool/min)

The formula has been developed assuming the summation of insulin from the peripheral circulation to be a first order process. In the present study it was 1 to 8 minutes. A k value of 0.1 was used corresponding to half-life ($T_{1/2}$) of insulin of about 7 minutes. For practical purposes the formula has been developed to (162)

$$ER = \frac{0.1 \times \Delta}{0.8}$$

where Δ is the insulin concentration from the basal level at 8 minutes and 0.8 is the value with insulin above the basal level. The value was calculated by approximating the insulin response with the straight line between the points 0, 4, 6 and 8 minutes.

Data from insulin analysis were missing in one of the 4, 6 or 8 minute samples in seven women. The concentration was calculated by intrasubject interpolation.

The estimation of the magnitude of the early insulin response will be further discussed in chapter IV. The assumption of this is that the results presented in that chapter will be of importance for the choice of method used to interpret the response.

The degree of body weight expressed as relative body weight calculated as a percentage of ideal weight obtained from a height weight table (112). Body weight and height of women not participating in the IVGTT were measured at the primary examination (n = 20) as was obtained from questionnaires of telephone interviews (n = 31).

The method used for dietary history and body composition in the study of dietary habits will be presented in chapter VII.

Comments

The high interindividual variation in the glucose tolerance test was determined by the need for an intervention in glucose load for the study of the early insulin response. Although the oral way of administration of glucose is more physiological the glucose disposal rate at fasted state now glucose load has been considered to be a convenient measure of a subject's ability in disposing of a glucose load. Comparatively, usually, however, shown a high incidence of disagreement when the diagnosis of diabetes made on the basis of an oral glucose tolerance test compared to an intravenous glucose tolerance test, as demonstrated by Olfky et al (127). It is obvious that the two methods will give different information about a subject's ability to act during a period of hyperglycemia. Which method is more reliable for prediction of future development of manifest diabetes needs elucidation by means of long term prospective studies. The methodological background for the method used in the present study has been carefully discussed by Lunell (117), Pehkonen (137) and Wahlberg (166).

The theoretical and experimental background for the method used to calculate the magnitude of the early insulin response (ER) has been presented by Thell et al (162) and will be further discussed in chapter IV.

Statistical methods

Conventional methods were used to estimate mean value standard deviation (S.D.) standard error (S.E.) and correlation coefficient (r). By means of the χ^2 transformation according to Fisher it was tested whether the correlation coefficient significantly differed from zero (20). Stepwise multiple regression was applied to investigate the linear dependence between $\ln k$ value and ER respectively and G_{dos} , G_{incr} , G_{stim} and initial body weight (53). One-way analysis of variance in some calculations combined with Scheffé's method for multiple comparison was used to test the hypothesis concerning the expected values in several populations (F) (10). The hypothesis concerning expected values in two populations was tested by means of the Student's t test (1). The hypothesis concerning difference in frequencies between populations was tested by means of the chi-square test (χ^2) or by means of the binomial distribution with a normal approximation (1) (20).

The difference was considered statistically significant if $p < 0.05$ which did not state the probability of obtaining the actual sample outcome or a more extreme result under the assumption that the stated null hypothesis is true. The data were analysed on a computer (IBM 360/65).

Roman numeral refers to the chapter of the monograph. Arabic numeral refers to the line number. pp 115-116

PREVALENCE OF MANIFEST DIABETES IN A POPULATION SAMPLE OF WOMEN AGED 50 AND IN THEIR FAMILIES

Göran Blohmé

The generally accepted view is that clinically manifest diabetes constitutes only a small part of the diabetic syndrome which also includes minor degrees of impaired glucose tolerance and the so-called prediabetic state (51). Studying women aged 50 and the low frequency of manifest diabetes may be expected as the prevalence in the general population in Sweden at this age has been estimated to be about 2 percent (72). The present population study was however mainly designed to study the background of the diabetic syndrome before the manifestation of the disease has appeared. A high prevalence of manifest diabetes is strongly gender-dependent (52-72) and an improvement of the total diabetic predisposition might be gained by studying the parent of the selected women. The main purpose of the part of the present investigation was to study the prevalence of manifest diabetes in the women randomly selected for the present population study and in their parents.

MATERIAL

Information concerning family history of diabetes was obtained from 98 percent (n = 426) of the total sample of women aged 50. The women had died and followed away from the town between the sampling and the examination date. Information concerning family history was thus obtained for 99 percent of the women available for study (n = 429). In women who did not know about the relative's family history were classified as "no". One pair of twins was present in the sample. The parents were counted for only one. A few women could not give information on the age of their parents at the present time regarding the information in this part of the present study was thus obtained from 97 percent of the women available for study concerning mother and for 95 percent concerning father.

For further details of the material see chapter I
 For methods and definitions see chapter I

RESULTS

Manifest diabetes in the population sample

Four of the women called to the primary examination had a previously known manifest diabetes. Two of them were being treated with insulin and two had been prescribed diet only. One woman with previously unknown diabetes had a fasting blood glucose concentration above 200 mg/100 ml on repeated analysis. In the group of women who refused to participate in the first examination none was aware of manifest diabetes. Death certificate disclosed that one of 15 women who died in 1950 or later (after the age of 32) had manifest diabetes. The frequency of manifest diabetes in women who were or would have been sampled for the age strata of 50 was 1.3 per cent.

Manifest diabetes in the family

The prevalence of known family history of diabetes was 30.4 per cent in women available for study (Table I). The women reported diabetes in mother about twice as often as in father (χ^2 12.2 $p < 0.001$). Three of five women with manifest diabetes reported a mother with diabetes. The prevalence of diabetes in parents who had died passed the age of 75 was 15.5 per cent in mothers and 7.0 per cent in fathers (χ^2 7.0 $p < 0.01$). In the group of parents 53 per cent of the mothers and 36 per cent of the fathers were living at the time of the study.

Table I Prevalence of family history of diabetes in women age 50 as a family study

Type of family history	n	%
Close familial history	80	18.7
Mother	49	11.4
Father	21	4.9
Siblings	10	4.2
Other	50	11.7
Eight women reported family history in both parents and siblings		

Th pa nt ag

The mean age of mother at the time of the study was 71.3 ± 12.4 (S.D.) years compared to 70.3 ± 14.1 (S.D.) years in father (t test $p > 0.1$). At the time of the study 63 percent of the mother and 79 percent of the father were dead some of them having died at a young age (Table II). About half of the parent had each died at the age of 75 years. A significantly high frequency of fathers had died before the age of 60 (26 percent $p < 0.01$) as well as before the age of 70 (23.5 percent $p < 0.01$) compared to the mother. The majority of the parental death was ascribed to infectious disease malignant disease accidents suicide. The frequency of death ascribed to heart disease was not significantly high in father compared to mother before the age of 60 (16 percent $p > 0.05$) before the age of 70 (Table III).

Table II. Data on parent of women aged 50 available for study (n = 428). The age of living parents was noted at the time of study. If a parent was dead the age at death was noted. MD missing data.

		Age (years)	<50	50	60	70	75	80	85	90
				59	69	74	79	84	89	94
Mother n = 416*	Alive (n)				2	48	53	35	10	5
	Dead (n)		26	30	65	51	48	27	16	
	Debatable (n)		1	1	7	10	17	9	3	1
	(%)		3.8	3.3	10.4	10.1	16.8	14.5	11.5	20.0
Father n = 405**	Alive (n)					14	35	25	8	3
	Dead (n)		40	42	67	43	55	58	11	4
	Debatable (n)		1	3	3	0	3	9	1	1
	(%)		2.5	7.1	4.5	0	3.3	10.8	5.3	14.3

MD 12

** MD 23

Table III Number of deaths ascribed to heart disease before the age of 70 in parent of women aged 50 (those specifically ascribed to myocardial infarction or angina pectoris also separately in brackets)

Age (year)		<50	50-59	60-69
Mother	n	2 (0)	5 (4)	21 (12)
Father	n	5 (2)	9 (7)	13 (11)

DISCUSSION

The prevalence of manifest diabetes in the present random sample of women aged 50 was 1.3 per cent. Although the material is too small to permit valid conclusions concerning the prevalence of diabetes in the general population at this age the result is consistent with that of a serial investigation in 1965 in Sweden (72). In that study the age-gestational morbidity risk for diabetes up to the age of 50 was estimated to be about 2 per cent in both sexes. In addition the prevalence of manifest diabetes in men aged 50 in Gotborg, Sweden was found to be 2 per cent (60).

The prevalence of manifest diabetes in Sweden is consistent with prevalence figures reported from other western countries (62-100 %). The prevalence of manifest diabetes has been reported to be as high as in some populations of so-called "Arctic Indians" (121) and Indians in South Africa (27). This indicates that local differences in genetic and environmental factors may be of considerable importance. The solution of the diabetes problem. Conclusions drawn from the data in the present study may thus only be valid for populations comparable to the ecological and socio-economic level as in Sweden and similar western countries.

A genetic predisposition to the development of diabetes mellitus has long been taken for granted. Diabetes frequently portends a family history of diabetes (reviewed by Hall and (82)). The reported prevalence of family history of diabetes is high in diabetes mellitus and diabetes (45-99 % (34, 159, 169)). A high prevalence of newly detected diabetes mellitus by itself with than in subjects with a family history of diabetes mellitus has also been found in a large study (83).

investigation (52-150) The most interesting point in the epidemiological figure is however that even individual without known diabetes about 98 per cent of the general population in western countries have a high prevalence of latent with diabetes. This is consistent with the total prevalence of 30.4 per cent in the present study mainly comprising women without manifest diabetes. This underlines the fact that the average prevalence of diabetes in the total population (1.2 per cent) does not fit the high total morbidity of the diabetes due to the fact that the prevalence is strongly age-dependent (52-72).

In the present study as many as 11.4 per cent of the women posed a diabetic mother and 4.9 per cent a diabetic father. Many of the non-diabetic parents had died at age when the prevalence of diabetes is rather low. Furthermore about 1/3 of the mothers and 1/5 of the fathers were still living at the date of the study and may develop diabetes later on.

The aggregate morbidity risk and life table expectation for clinically manifest diabetes in Sweden in 1965 was calculated by Gronberg et al (72). The aggregate morbidity risk for women in a study from 19 per cent at the age of 50 to 10.5 per cent at the age of 75 and to 13.0 per cent at the age of 90. The corresponding figures for men were 2.1, 5.3 and 6.5 per cent respectively. Thirteen per cent for women and 6.5 per cent for men present the total morbidity risk of an individual developing manifest diabetes up to the age of 90. However many of the presumptive diabetics will not develop the disease as they will die before the onset of the disease. An explanation of the probability that a newborn child will develop manifest diabetes during his lifetime is derived at by determining the life table expectation for manifest diabetes. The life table expectancy was calculated to be 19.92 and 10.2 per cent for women and 20.44 and 4.8 per cent for men at the age level mentioned (72). In the present study in the whole had reached the age of 75 had a prevalence of diabetes of 13.5 per cent compared to 7.0 per cent in fathers. In spite of the fact that about 1/2 of the mothers and 1/3 of the fathers in this group were still living. The total morbidity could be expected to be a further if the material had been examined more recently. The disposition for diabetes in patients who had died before the age of 75 in for example diovascular disease might well have been still high as the free

quency of impaired glucose tolerance by definition chemical or subclinical diabetes (51) has frequently been reported to be high in patient with such disease (86 135 166) A high frequency of early death in fathers than in mother found in the present study was mainly due to causes other than diabetes however The higher prevalence of diabetes in mothers than in fathers is consistent with results obtained in other recent studies (52 72 107) indicating a high diabetic predisposition in women than in men

A limitation of the study is conclusion concerning the prevalence of a family history of diabetes in the present study have been based on information gained by interviews or questionnaire among the probands Some error other than those discussed above may thus have been introduced in the calculation of the total morbidity risk for manifest diabetes A great variability of the disease pattern with a high proportion of mild cases especially in the elderly would explain the high proportion of previously unknown cases (30 40 per cent) found in the program studies for diabetes (52 145 169) In the present study none of the diabetic patient was previously known Reporting of diabetes among relatives depends on the proband knowledge of the disease history of the relatives In the population study in T. Malm the family history data for sibling which included a subgroup (124) The number of follow-up data was negligible The subjects interviewed knew how of only 25 per cent of the actual number of cases of diabetes in the sibling

The type of reproduction and estimation of the actual frequency of manifest diabetes and might cause marked underestimation of the total morbidity risk for the disease Therefore the authors would like to suggest that the total morbidity risk for diabetes in Sweden will exceed the 13 per cent for women and 6.5 per cent for men reported by Gönberg et al (72)

SUMMARY

The prevalence of manifest diabetes in women aged 50 who were selected for the present population sample would have been limited if still alive was 13 per cent Of 100 women had previously unknown diabetes The prevalence of a known family history of diabetes was 30.4 per cent 1. The prevalence of diabetes in mothers (11.4 per cent)

was twice that in fathers (4.9 per cent). Mothers who had reached the postmenopausal age of 75 had a prevalence of diabetes of 15.5 per cent. The diabetic predisposition in women will be rather high as the total morbidity risk for diabetes in women may be assumed to exceed the 13 per cent suggested by Gombertz (72).

Chapter III

INTRAVENOUS GLUCOSE TOLERANCE IN A POPULATION SAMPLE OF WOMEN AGED 50

Göran Blohmé

Population studies in Bedford (25) Tecumseh (83) Sudbury (134) parts of southern California (146) Greater Cleveland (104) and other areas have indicated that hypoglycemia during oral glucose tolerance tests is a not uncommon finding in the general population especially in older individuals. Differences in composition of studied group in method of testing and in the criteria used for evaluation of results may be responsible for the differences in reported prevalence figures caused by O'Sullivan (130). The nationwide prevalence of hypoglycemia in the U.S. has been estimated using the results of a health examination survey conducted by the National Center for Health Statistics on a representative sample of the U.S. adult population (122). In the age group 45-54 8.2 per cent had a blood glucose concentration above 180 mg/100 ml one hour after ingestion of 50 g of glucose. In the age group 75-79 the prevalence was higher as 29.5 per cent.

The intravenous method for determining the tolerance to glucose has mainly been used in limited groups of subjects. This circumstance is a unique disadvantage for mass screening. No thorough study of healthy nondiabetic individuals has been studied in Sweden during the years using the same method (85, 117, 166). Only one of them however was performed in subjects randomly selected from the general population (85). In this study only minor results had to be said about the prevalence of an impaired tolerance to an intravenous glucose load in middle aged women randomly selected from the general population.

The main purpose of this part of the present investigation was to study the frequency distribution of the glucose disappearance rate of the fasting individual using the standard stimulus of 25 g of intravenous glucose tolerance test.

For material and methods see chapter I

RESULTS

Fasting blood glucose

Fasting venous blood glucose concentration tended to be normally distributed in women without manifest diabetes with a mean value of 70.2 mg/100 ml (Table I). A weak correlation was found between fasting blood glucose concentration and relative body weight ($r = 0.28$, $n = 37$, $p < 0.001$). A significant increase in fasting blood glucose level with increasing relative body weight was also shown using one-way analysis of variance (Table II).

Table I Blood glucose concentration before and after an intravenous glucose load in 352 women aged 50. G_{inc} mean of increment in blood glucose at 4 and 6 minutes. G_{tim} mean of increments per minute during the first five minutes.

	Blood glucose mg/100 ml		
	Mean	S.D.	Range
\bar{x}	70.2	8.3	52-107
4 min	320.2	53.0	139-465
6	323.4	34.6	205-414
8	303.3	30.9	211-451
G_{inc}	251.6	37.9	120-363
G_{tim}	150	29.1	44-231

Glucose stimulation

A wide range in blood glucose concentration was observed after the intravenous glucose load. This was evident in the total material (Table I) as well as at different levels of relative body weight (Table II). The glucose stimulation expressed as G_{inc} and G_{tim} thus varied markedly between the women. A significant increase in the variance was noted with increasing relative body weight using one-way analysis

Table II. Fat, blood glucose and blood glucose stimulation in 352 women subgrouped according to
 1. body weight 2. symbols table 1

Relative body weight	n	Fat in blood glucose		Glucose		Glucose		Glucose	
		Mean	S D	Mean	S D	Mean	S D	Mean	S D
		mg/100 ml		mg/100 ml		mg/100 ml		mg/100 ml	
90	34	66.8	8.3	1.8	34.8	141.297	131.1	27.2	64.173
91-100	77	68.4	6.9	238.1	34.9	137.319	142.6	27.1	79.195
101-110	106	69.4	7.5	250.6	37.3	120.348	149.0	30.3	44.231
111-120	70	71.9	8.3	61.1	33.9	164.327	157.0	26.0	89.212
121-130	31	73.5	8.5	64.1	27	194.316	156.5	4.0	99.02
131	34	73.8	10.8	83.9	32.9	19.363	170.5	25.6	1.030
F _{ob}		5			14.3			9.3	
P		< 0.01			< 0.01			< 0.01	

of variance (Table II) as well as regression analysis ($P = 0.43$ and 0.34 , 8.59 and 6.62 respectively $P < 0.001$)

Glucose disposal and rate

The distribution of k values was skewed but tended to be normalised after logarithmic transformation (Fig 1). There was no evidence of a bimodal distribution of the k values. The distribution of k values was the same whether the material was restricted to women with a relative body weight of ≤ 120 per cent ($n = 287$) or a relative body weight of $91-110$ per cent ($n = 183$).

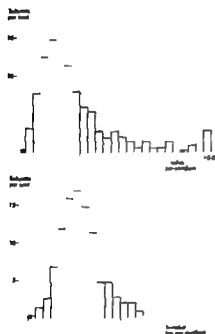


Fig 1. Probability distribution of the k values and the logarithms in 352 women aged 50.

The k values varied over a wide range with a median value of 1.88 and a median value of 1.59 . A few women with extremely high glucose disposal rates had already checked their fasting blood glucose level within 30-35 minutes after the start of the glucose injection. The median $\log k$ value was 0.2218 corresponding to a k value of 1.67 . The distribution of the distribution of the k values is presented in Table III. Applying the distributional limits for normal and pathological k values it was found that 6.0 per cent of the women had a k value below 0.90 , 11.1 per cent a k value below 1.00 and 17.3 per cent a k value below

1 10 When the material was restricted to women with a relative body weight of ≤ 120 per cent the corresponding frequencies were 6 3 10 5 and 15 0 per cent respectively. In women with a relative body weight of 91 110 per cent 4 9 9 8 and 15 8 per cent had a k value below the limit.

Table III Decentile of the k value distribution in 352 women aged 50

Decentile	k value	Decentile	k value
1	0 97	6	1 75
	1 13	7	1 97
3	1 29	8	2 29
4	1 44	9	3 13
5	1 59		

Correlation

Correlation coefficient near zero was found between the k value and the glucose stimulation expressed as G_{incr} ($r = 0 04$ \pm $0 75$ $p > 0 1$) and G_{sum} ($r = 0 05$ \pm $0 94$ $p > 0 1$) respectively. Only 1 4 per cent of the total variance in the k value was explained by the glucose dose which in fact was weakly negatively correlated to the k value ($r = -0 12$ \pm $0 25$ $p < 0 05$) (Fig. 2).

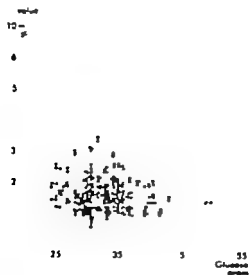


Fig. 2 Relationship between administered total dose of glucose and k value in 352 women aged 50

The G_{in} and G_{turn} varied markedly even in women with adjacent body weights and relative body weight. In a group of women with relative body weights of 95-105 percent ($n=110$) those with a body weight of 60.0-64.9 kg were selected ($n=47$). In this homogeneous group as regards total dose of glucose and dose of glucose per kg fat free weight the G_{in} ranged from 151 to 330 mg/100 ml and the G_{turn} from 77 to 210 mg/100 ml. No significant correlation was found in this group between G_{inc} and the k value ($r=0.24$ $p>0.1$) or between G_{turn} and the k value ($r=0.22$ $p>0.1$).

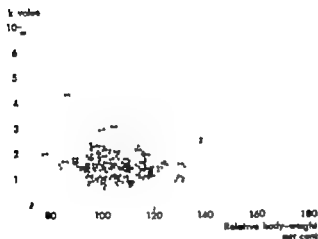


Fig. 3 Relationship between relative body weight and k value in 352 women aged 50

A very weak correlation was found between relative body weight and the k value ($r=0.11$ $p<0.05$) (Fig. 3). The tendency of increasing k value with increasing relative body weight was further shown when the women were grouped according to relative body weight (Table IV). The tendency of increasing k value with increasing relative body weight was, however, not significant using one-way analysis of variance. No could the chi-square method disclose any dependence of k value on relative body weight when tested on the whole material (Table V). The frequency of k value below 1.10 was, however, significantly high in women with a relative body weight below 120 percent than in lean women ($\chi^2=2.44$ $p<0.01$). In addition the frequency of k value above the last (ninth) decile of the distribution of k values was significantly low in women with a relative body weight below 120 percent than in lean women ($\chi^2=2.82$ $p<0.01$).

Table IV The k value in the total material and subgrouped according to relative body weight

Relative body weight %	n	k value		
		Mean	S D	Range
76-170	352	1.88	1.10	0.59-9.86
≤ 90	34	2.01	1.12	0.62-5.62
91-100	77	1.86	0.95	0.59-6.42
101-110	106	2.08	1.37	0.71-9.86
111-120	70	1.71	0.79	0.66-4.77
121-130	31	1.74	1.25	0.85-6.70
≥ 131	34	1.67	0.70	0.77-3.72
F _{ob}		1.5		
p		> 0.05		

Table V Vomition (%) in the total material and in classes of relative body weight subgrouped according to the k value

Relative body weight %	n	k value						
		< 0.90	0.90-0.99	1.00-1.09	1.10-1.19	1.20-2.29*	2.29-3.12*	≥ 3.13
76-170	352	6.0	5.1	6.3	6.3	56.3	9.9	10.2
≤ 90	34	5.9	2.9	2.9	8.8	52.9	11.8	14.7
91-100	77	5.2	6.5	9.1	3.9	53.2	14.3	7.8
101-110	106	4.7	3.8	3.8	10.4	52.8	9.4	15.1
111-120	70	10.0	2.9	1.4	5.7	64.3	10.0	5.7
121-130	31	6.5	9.7	12.9		61.3		9.7
≥ 131	34	2.9	8.8	14.7	2.9	55.9	8.8	5.9
χ ²		36.1						
p		0.1						

k value total VIII 293

k value total IX 316

A stepwise multiple regression analysis was performed to evaluate the compound influence of latitude, body weight, glucose G_{in} and G_{tum} on the k value (Table VI). These four variables together could only explain 2 percent of the total variance in k value.

Table VI Stepwise multiple regression analysis with k value as the dependent variable. For symbol of abbreviations, see Table I. Variables listed in the same order they are entered into the regression equation. b (coefficient of G), β (coefficient of latitude), β (coefficient of standard regression) are final adjusted R^2 (function of explained variance) and $s.e.$ (standard error).

Variable	b	β	R^2
G_{do}	0.011	0.06	0.0140
G_{tum}	0.010	0.28	0.0143
G_{in}	0.001	0.29	0.0193
Latitude body weight	0.006	0.09	0.0203
Coefficient of multiple regression	0.14		
F test for explained	0.02		
Constant term	2.39		

DISCUSSION

The present study showed a wide range of glucose disposal rates in the postprandial state. The glucose disposal rate may change from time to time with the same individual (12, 56, 84, 135, 137, 166). The glucose disposal rate may be related to the amount of circulating insulin as well as to the amount of insulin sensitivity in peripheral tissues. Several hormones will directly or indirectly be involved in this connection (89). A detailed description of the factors involved in the regulation may be needed to minimize such factors.

possible. As emphasized by Fajns (64) however, the lack of reproducibility of an oral glucose tolerance test in cases of borderline glucose tolerance may impact on the clinical history of diabetes with fluctuations in the degree of disturbed glucose homeostasis. No representative waveform was formed in the present investigation due to the fact that the women studied had not taken part in numerous studies and furthermore, a planned follow-up. It may well be that some of the women with a low HbA_{1c} would have shown an improvement in glucose tolerance if a repeated test had been performed while other women would have shown a deterioration.

The frequency distribution of the HbA_{1c} was skewed with the slope towards higher values which is consistent with results obtained in other studies (11, 116, 117, 166). The continuous and unimodal distribution of the HbA_{1c} in the present study as well as in previous studies indicates that it is impossible to establish a limit between increased tolerance to glucose and normal tolerance except arbitrarily. Unimodal distribution of the blood glucose level after an oral glucose load has also been reported (19, 25, 83). The lack of bimodality has often been taken as evidence for a multigenic nature of the diabetic predisposition. This hypothesis has been discussed and challenged by Stenbäck (155). In populations with a very high prevalence of impaired tolerance to glucose demonstrated globally a bimodal distribution of the two-hour glucose level has been reported (156). The interpretation of the findings is that the diabetic predisposition might be due to a mixture of the general population might be composed of two subpopulations, normal and one diabetic, a diabetic population but would be distributed where only a small proportion consisted of diabetic patients. The unimodal distribution of the HbA_{1c} in the present study and the uniform high diabetic predisposition (11) do not support the hypothesis that a special population with a genetically determined predisposition for diabetes would be characterized by a markedly low glucose disappearance rate than individuals without the predisposition.

The mean HbA_{1c} (1.88) in the present study is generally well within the normal range (1.91 mean \pm 0.50 normally, slightly different from the population in Lappeenranta 1.54 (85). It is higher than the mean value of 1.58 which was found in 348 (mean \pm 0.55) in the study by Wahlberg (166). In the study of Wahren \pm 0.36 (mean \pm 0.46) by L. 11

(117) the mean k value was calculated from log k value. The mean log value in this study corresponding to a k value of 2.14 was higher than the mean log k value of the present study corresponding to a k value of 1.67. The result of some other studies have been reviewed by Wahlberg (166). Reported differences in mean k value might depend on differences in composition of studied group as well as different type of procedure. Several factors might be considered to influence the glucose disappearance rate from which will be discussed further.

Carbohydrate feeding. The use of carbohydrate rich diet before the performance of a glucose tolerance test has been subjected to discussion for decades as reviewed by Lunell (117) and Wahlberg (166). Recent studies have again shown that carbohydrate feeding may improve and carbohydrate tolerance test in normal subjects as well as in subjects with impaired glucose tolerance (6, 21, 89). Limited data on the effect of change in dietary carbohydrate on the glucose disappearance rate after an intravenous glucose load suggest a small influence on this parameter (6, 47, 89, 109, 166). This effect has been attributed to altered sensitivity to insulin in peripheral tissues when the consumption of dietary carbohydrate is changed (89).

A population of subjects with a carbohydrate content of about 300 g was prescribed during 3 days before the test in the present study as a diet restricted in carbohydrate may be a common finding in middle aged women especially during a weight reducing regimen. It cannot be excluded that some of the women with a body mass index of low normal k value in the present study would have had k value within the pathological range without carbohydrate feeding. In this study by Lunell (117) carbohydrate rich diet was prescribed before the test while in the study by Wahlberg (166) and Heden and (85) no carbohydrate preloading was used.

Age. A decreasing k value has been reported with increasing age in healthy subjects (11, 44, 117) as well as in patients with diabetes (135, 166). This decrease has mainly been attributed to an increasing degree of obesity with age (116, 117). A marked decrease in mean k value was however noted when a group of women in the study of Lunell (117) were tested 5-7 years later at a mean age

of 52 diabetic patients unchanged mean body weight (118). This indicates that aging process may be associated with a decreasing rate of glucose utilization which might depend on decreasing sensitivity to insulin in peripheral tissues with age. In the present study the age factor was removed as only on age patients were studied.

Obesity. It is generally pointed out that obesity is strongly associated with the development of mature onset diabetes (52, 101, 164, 168). In the present study a tendency to decreasing k value with increasing relative body weight was noted. This was mainly attributed to a high frequency of k value below 1.10 and low frequency of high k value in women with a relative body weight above 120 percent compared to lean women. These observations found between obesity and a decreasing k value in agreement with results obtained in other studies (11, 85, 116, 117, 137) and may be due to a decrease in insulin sensitivity per peripheral tissue with an increasing degree of obesity as discussed by Horton et al. (89).

Glucose stimulation. In the present study, in that of Hedt and (85) the glucose dose was adjusted according to body weight as suggested by Dik and Luft (91). The glucose dose per kg fat free weight then varied within a range of obesity. The glucose stimulation was thus high in obese than in lean women. In the present study this was reflected by an association although weak between relative body weight and the increase in blood glucose concentration during the first few minutes after the glucose injection. Considerable individual variations both in lean and obese women in blood glucose increment after the glucose injection were found. A wide range of blood glucose increment was also found in women with body weight within a 10% range and relative body weights within ± 5 percent of ideal weight. The present results obtained in the present study only reported studies of the glucose dose with a column of data but of glucose (32%) of significant interest was found between glucose stimulation and the k value. However, the relationship between the peripheral glucose disposal rate and glucose dose per kg fat free weight.

Although the relationship was reported with the glucose dose was determined as 50 g/min in the present study.

(55-166) In other studies however, an increase of the glucose dose had no significant influence on the k value (5-42-117). In the present study the glucose dose was 25-40 g in 90 per cent of the women. The regression analysis showed a very weak negative correlation between the glucose dose and the k value as well as between the relative body weight and the k value. These two variables could each explain only about 1 per cent of the total variance in k value. It cannot be excluded however that the high dose of glucose in obese women is than in lean women might have caused somewhat higher k values in the obese women than if a standard dose of 25 g had been used. This tendency to a decreasing k value with increasing relative body weight may thus have been underestimated. The stepwise multiple regression analysis showed however that the effect of obesity on the k value if glucose stimulation was the major determinant of the k value in the present study. In fact the variable t_g could only explain about 2 per cent of the total variance in the k value.

Predictive value of the k value in the diagnosis of diabetes

Based on studies in patients with clinically manifested diabetes a k value below 1.00 has frequently been regarded as a pathological value evidenced by Wahlberg (166). The limit was chosen empirically. Wahlberg (166) found the mean k value plus two S.D. in a group of patients with clinically manifested diabetes to be 0.91. In a view of this limit, Wahlberg found that 7 per cent of patients with diabetes had a k value of 0.91 or higher. The limit of the diabetic patient and the unimodal distribution of the k value in the control subjects without diabetes have not been used. Wahlberg's criterion to define k values as below 0.90 is perhaps also between 0.91 and 1.10 a borderline and above 1.10 a normal (166). In the present study 60 per cent of women had a k value below 0.90 and 11.4 per cent a k value between 0.90 and 1.10. This frequency was essentially the same when the men were related to women with a relative body weight also below 120 per cent within ± 10 per cent of ideal weight. In the study of randomly selected men aged 50 in Uppsala, Sweden the corresponding frequencies were 9.5 and 4.3 per cent respectively (85). In the study by Wahlberg (166) 6 per cent of men and only one per cent of women had a k value ≤ 0.90 while the borderline frequency was 12 and 7 per cent respectively. In Lunell

study (117) none had a k alu < 1.00 . When ret ting a subgroup five years later 7 percent had developed a k value < 1.00 however (118). It is obvious that factor discussed above concerning differences in composition of studied group and in test procedure might explain the divergent results.

The result of the present study indicates that a considerable number of women aged 50 have a glucose disappearance rate which is generally accepted to indicate a diabetic state. The significance of a low k value and of such factors as obesity, dietary habits and family history of diabetes for the future development of manifest diabetes needs a prospective study to be determined.

SUMMARY

The present cross sectional study of women aged 50 showed a wide range of diastolic pressure with a mean value of 1.88 and a mean diastolic value of 1.59. The continuous and unimodal distribution of the diastolic value did not permit to establish a limit between normal and a normal value except a bit arbitrarily using a theoretical limit as a considered number of women having a diastolic value which is generally accepted to indicate a diastolic value. Sixty percent of the diastolic value < 0.90 11 percent of the diastolic value < 1.00 and 17 percent of the diastolic value < 1.10 . A weak association was found between obesity and a diastolic value. The value of the latent body weight together with the mean diastolic value stimulated could however only explain 2 percent of the total variance in the diastolic value.

EARLY INSULIN RESPONSE IN A POPULATION SAMPLE OF WOMEN AGED 50

Göran Blohmé and Johan Waldenström

A considerable amount of work has been performed during the last twenty years in the study of the insulin secretory capacity as reflected by an increase in insulin concentration in the peripheral blood especially in individuals with minor degrees of abnormality in the glucose homeostasis with a genetic predisposition for diabetes mellitus as reviewed by Kipnis (105) and Peto and Bagdad (138). Interestingly, mainly been focused on the morphology of the secretion of insulin as a result of the hypothesis being put forward that a delayed and delayed early insulin response to glucose stimulation might be the basic pathogenic factor in the development of all types of the diabetic syndrome including the pre-diabetic state (35). There is no general agreement, however, on this hypothesis (94, 141, 159). The frequently small size of highly selected subjects differing in methods of glucose stimulation and technique of evaluation of the insulin response upon might well be factors responsible for this confusion.

The high prevalence of diabetes in the general population especially in women (11) clearly indicate that a method of evaluating the predisposition would be of considerable importance in the early diagnosis and prevention of the disease. In 1968 when the present study was started no information was available about the distribution of the early insulin response in the general population. Preliminary data have recently been reported however from a population study of men in Uppsala, Sweden (85).

The main purpose of this part of the present investigation was to study the frequency distribution of the glucose stimulated early insulin response as reflected by an increase in insulin concentration in the peripheral blood in a random sample of women aged 50 and to evaluate to what extent factors such as glucose stimulation and relative body weight influence the magnitude of the response. In the following chapter

study (117) none had a k value < 1.00 . When retesting a subgroup five years later 77 per cent had developed a k value < 1.00 however (118) other statistical factors discussed above concerning differences in composition of studied group and in test procedure might explain these divergent results.

The results of the present study indicate that a considerable number of women aged 30 have a glucose disappearance rate which is generally accepted to indicate a diabetic state. The significance of a low k value and of such factors as obesity, dietary habits and family history of diabetes for the future development of manifest diabetes need a prospective study to be determined.

SUMMARY

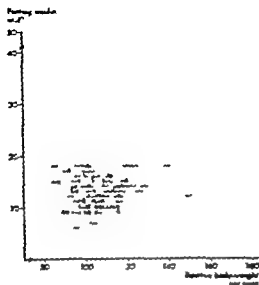
The present cross sectional study of women aged 30 showed a wide range in glucose disappearance rate with a mean k value of 1.88 and a median k value of 1. The continuous and unimodal distribution of the k values do not permit to establish a limit between a decreased and a normal k value except arbitrarily. Using traditional limits a considerable number of women showed a k value which is generally accepted to indicate a diabetic state. Six per cent had a k value < 0.90 , 11 per cent a k value < 1.00 and 17 per cent a k value < 1.10 . A weak association was found between obesity and a decreased k value. The increase in body weight together with the increase in glucose tolerance could, however, only explain 2 per cent of the total variance in the k value.

T b J S rum sul n n nt t n b f and ft int v n u gl o lo d l n th t tal m t al of 352
 worn ged 50 and in worn bg ouped o d g t l t body w fght

R latu body w (%)	n	F u g	In ul w (mU/l) 1)					8 min					
			4 min	6 m	6 m	6 m	6 m						
76 170	352	13 9 5 3	3 47	86 7	46 8	1	390	72 6	45 6	12 495	60 9	37 4	14 380
< 90	34	11 9 3 8	5 19	82 9	37 3	20	185	66 5	30 3	20 137	53 4	4 4	16 11
91 100	77	12 4 4 0	3 2	72 5	31 1	2	38	58 8	25 1	1 180	47 5	20 0	14 136
101 110	106	13 8 5 7	6 47	92 2	55 1	12	390	77 5	57 5	14 495	64 7	45 1	14 380
111 110	70	14 7 5 3	7 36	93 1	13	8	45	77 5	38 5	27 230	64 8	33 9	23 195
121 130	31	15 1 5 0	7 30	84 8	46 9	26	184	76 5	47 2	0 18	66 8	1 7	22 180
< 131	34	16 7 6	7 31	93 9	58 2	29	345	80 7	58 1	27 340	74 1	45 2	38 260
F b		5 06	4					2 25			3 78		
P		< 0 01	0 05					< 0 05			< 0 01		

1) Mean S D and Rang

Fig 2 Relationship between relative body weight and fasting insulin concentration in 352 women aged 50



Insulin secretion

The intravenous glucose load elicited an increase in the serum insulin concentration in all but one woman during the first 8 minutes after the start of the glucose infusion. A considerable range was found in insulin concentration reached during the period studied in the total material as well as at different levels of relative body weight (Table 1). Obviously men showed a significantly higher insulin concentration than did the women when tested by conventional analysis of variance. When the t -test was applied to the mean insulin concentration from the two last 4, 6 and 8 minutes the increment did not differ significantly with increasing relative body weight except after 8 minutes. For the 8 minute increment $3.05 \mu U/L$ ($P < 0.05$). The peak in insulin concentration was noted at 4 minutes in 86 per cent and at 6 minutes in 11 per cent of the women. A peak value below 10 mU/L was noted in 4 per cent (n 8), and above 200 mU/L in 1 per cent (n 4) of the women.

Early and late postprandial (ER)

In the present study the mean incremental insulin concentration after 4, 6 and 8 minutes was related to all values of the total early insulin

upon (ER) according to the total (162) ER was clearly related to other measures of the insulin in the example the increase at 4 minutes (0.95) in the case at 8 minutes (0.99) and the area below the insulin curve above baseline from 0 to 8 minutes (0.99). In the following we will use only the ER value which will be taken into account.

The range in ER was considerable (1.559 mU/l) with a mean value \pm S.D. of 85.4 ± 60.4 mU/l and a median value of 72 mU/l. The frequency distribution was skewed in the total material with the tail toward high values but tended to be normal after logarithmic transformation (Fig. 3). There was no indication of bimodal distribution of the ER variable. The distribution was the same when the material was restricted to women with a relative body weight ≤ 120 percent within ± 10 percent of ideal weight. The details of the ER distribution are presented in Table II. Only 2 percent (n = 8) of the women had an ER below 10 mU/l.

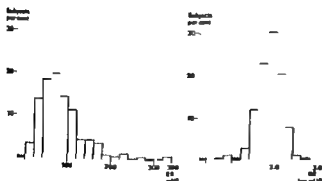


Fig. 3 Percentages of distribution of the logarithmically transformed (ER) and the logarithm in 352 women aged 50.

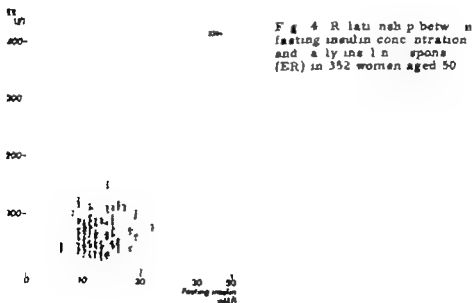
Table II Decentile of early insulin response (ER) in 35 women aged 50

Decentile	ER(mU/l)	D centile	ER(mU/l)
1	30	6	83
2	41	7	98
3	50	8	115
4	63	9	154
5	72		

The mean ER of 85.4 mU/l in the total material may be compared with a mean ER of 91.1 mU/l in a group of women with a relative body weight of 95-105 percent and a body weight of 60.0-64.9 kg (n=47) thus homogeneous as regards total glucose and glucose disposal/kg fat free weight.

Factors influencing the early insulin response

A weak correlation (r=0.26) was found between fasting insulin concentration and ER ($p < 0.001$) (Fig. 4).



The increase in blood glucose concentration during the first minutes of the test varied markedly and is discussed in chapter III. The correlation between glucose stimulation, a particular mode of exploration and ER were however very extremely weak (Table III). The weak correlation is illustrated in a scatter diagram where one of the variables of glucose stimulation G_{stim} is plotted against ER (Fig. 5). Women with a G_{stim} below the fitted constant of the G_{stim} variable had an ER which did not significantly deviate from ER in women with a G_{stim} above the high limit [79.7 \pm 45.0 mU/l and 92.0 \pm 60.0 mU/l (mean \pm S.D.) respectively $t = 0.97$ $p > 0.1$]. The correlation between glucose stimulation and ER was studied in the previously mentioned subgroup of women with a relative body weight of 95-105 percent and a body weight of 60.0-64.9 kg ($n = 47$). Neither in this subgroup of women was any significant correlation found between G_{inc} and ER ($r = 0.05$; $t = 0.33$ $p > 0.1$) or between G_{stim} and ER ($r = 0.05$; $t = 0.33$ $p > 0.1$).

Table III Coefficient of correlation in 352 women aged 50. ER = daily insulin pump G_{do} = total glucose G_{in} = mean fasting blood glucose at 4 and 6 minute G_{stim} = mean of increment per minute during the first five minutes

Variable	ER	r
G_{do}	0.10	0.87
G_{in}	0.12	0.25*
G_{stim}	0.08	0.50

* $p < 0.05$

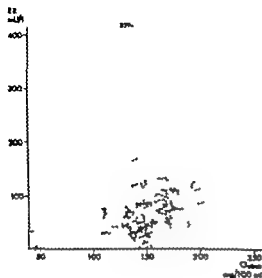


Fig 5 Relationship between glucose stimulation (G_{stim}) and early insulin response (ER) in 352 women aged 50

A weak correlation ($r = 0.14$) was found between the relative body weight and ER ($r = 0.14$, $p < 0.01$) (Fig 6). Furthermore, there was an ER in a statistically significant way with increasing relative body weight in women grouped according to relative body weight tested by one-way analysis of variance (Table IV). The variance in ER was more variable in the total material as well as in women grouped according to relative body weight with low variance in lean and high variance in obese women. No dependence was found between relative body weight and ER when the four-quartile test was applied to the total material (Table V). A high frequency of ER above the threshold level of the variability in the total material was however noted in women with a relative body weight above 100 percent compared to lean women ($p < 0.001$). The frequencies of women below the threshold and conditions were not significantly different between the groups of women ($p > 0.05$ and $p > 0.1$ respectively).

Fig 6 Relationship betw en
r l tiv body weight and
a ly in ulin spon (ER)
in 352 women aged 50

200-

200-

100-



Tabl IV Ea ly in ulin spon (ER) in 352 women
aged 50 sub grouped o ding to lati body weight

Rel tive body w ight (%)	ER(mU/l)			
	n	M an	S D	Rang
≤ 90	34	77.9	42.8	1-172
91-100	77	65.9	35.4	6-228
101-110	106	92.2	72.3	1-559
111-120	70	91.3	54.1	22-280
121-130	31	90.1	68.7	7-271
≥ 131	34	99.3	75.0	9-416

F_{ob} 2.57

P < 0.05

Table 4 Women (%) in the total material subgrouped according to relative body weight and early inulin response (ER)

Relative body weight %	n	Deciles of ER				
		I	II	VIII	IX	
≤ 90	34	8.8	14.7	61.8	5.9	8.8
91-100	77	15.6	7.8	68.8	6.5	1.3
101-110	106	7.5	13.2	55.7	9.4	14.2
111-120	70	8.6	8.6	58.5	14.3	10.0
121-130	31	12.9	9.7	48.4	12.9	16.1
≥ 131	34	8.8	2.9	64.8	8.8	14.7
χ^2		22.3				
p		> 0.1				

A stepwise multiple regression analysis was performed to estimate the fraction of total variance in ER which was due to the factor G_{do} , G_{in} , G_{tum} and late body weight. The fraction was estimated to be 3 percent.

DISCUSSION

Fasting insulin concentration

The distribution of fasting insulin concentration was skewed in the total material with the low to high concentration which coincided with previous reports (19, 39, 167). Obese women mainly, but due to the skewed distribution. This is in agreement with the normal distribution of fasting insulin concentration found in a group of normal young subjects with body weight of ± 10 percent of ideal weight (73).

The majority of women of normal weight are only moderately obese, although a weak association was found between fasting insulin concentration and degree of obesity. This is in agreement with the standard deviation of moderate obese patients as viewed by Rubinstein (140). Other groups have failed to show

this relation how various views by Olufsky et al (128) The fasting concentration of insulin is considered to depend on factors associated with the degree of insulin resistance in peripheral tissues (69 89 128 140) The adipose tissue has been discussed most frequently in this respect. Tissue other than the adipose tissue for example muscle have also been considered as the source of the insulin resistance however The weak correlation between fasting insulin concentration and relative body weight in women in the present study may indicate that tissue other than the adipose tissue may be the main determinant of the concentration of fasting insulin in lean and moderately obese women

Insulin secretion

The present investigation was undertaken to study the physiology of the glucose stimulated insulin response Glucose is many secretagogues capable of stimulating the β cells to secrete insulin as viewed by Mayhew et al (120) Kipni (106) and Malin (119) Studies in man have indicated that the glucose stimulated release of insulin is biphasic with an early rapid phase followed by a late slow and sustained phase (30 139 165) The biphasic response has also been shown in studies on the isolated perfused rat pancreas (24 46 71 90) indicating the existence of two distinct functional pools of insulin within the pancreas itself. The first of the pools is considered to be a secreted pool of limited size of readily available insulin The early rapid phase of the insulin secretion was estimated to account for only 2 per cent of the total extractable pancreatic insulin content in the rat in vitro (46)

If one considers the delay involved in the transport of glucose and inulin, the evidence suggests that the early release of insulin is almost instantaneous The inulin delivered to a peripheral site after the first and very little inulin will be released after 4-5 minutes from the pool (17 46 71 162 165) The late phase of the insulin secretion is considered to start within a few minutes of the start of the glucose stimulation (17 46)

Insulin concentration markedly higher in portal than in peripheral blood when measured simultaneously indicating a high removal of insulin by the liver reviewed by Feld (66) The relation between insulin concentration measured at the two locations indicates

however that the insulin concentration in peripheral blood reflects the secretory rate of the pancreas and may be used to evaluate the insulin secretory capacity (17-31)

In the present study the peak insulin concentration was noted at 4 minutes in 86 per cent of the women which is in accordance with other studies (147-148, 151-152, 162). In studies in which the insulin concentration was measured even earlier than 4 minutes a few subjects had each displayed a level even before that time (123, 162).

Early insulin response

Many methods have been devised to express the magnitude of the early phase of the insulin secretory response effected by an insulin concentration increase in peripheral blood. The disappearance rate of insulin from peripheral blood is higher than previously expected. The half-life ($T_{1/2}$) has more recently been calculated to be 3.7 minutes (30, 153, 160, 162, 165). In the present study the early insulin response (ER) was calculated according to Thorell et al. (162) using insulin levels at 0, 4, 6 and 8 minutes and a $T_{1/2}$ of insulin of 7 minutes. This method also takes into account the part of labeled insulin which disappears in the course of the test. ER will thus represent the increase in concentration of insulin that would have occurred if all insulin labeled into the general circulation had remained within the plasma pool (162).

A loss of correlation was however noted between ER and the minimal insulin concentration at 4 and 8 minutes as well as the early below threshold insulin concentration above baseline between 0 and 8 minutes positively. This indicates that results obtained with methods using the minimal peak value (165) or an average value (139-149) at a specific point of time (1, 50, 141, 147, 148, 152) and a value below the insulin concentration above the baseline (143) may be regarded as comparable if the blood samples have been taken during the first few minutes of the test with the first blood sample not later than 4-6 minutes after the start of the glucose injection. When the first sample has been taken at 10 minutes or later (30-94) it may have been introduced in the estimation as far as the early phase of the insulin secretion is concerned.

The present cross sectional study of women aged 50 showed a wide range in early insulin response indicating considerable differences in insulin secretion capacity. The reproducibility of the response to an intravenous glucose infusion was investigated by Cassi and Luft (33). They stated that the variation between repeated tests were surprisingly small. A high reproducibility of the early insulin response has also been found by Hedstrand and Bobe (84). No repeated tests were performed in the present investigation. A standardized test procedure including pre-treatment with a carbohydrate rich diet was used in order to standardize the experimental conditions as far as possible.

The frequency distribution was skewed with the slope toward higher values but continuous and unimodal. This is consistent with that obtained in men aged 50 (85) as well as in a group of young individuals (159). The unimodal distribution of the ER variability parallels the condition found when studying oral (25-83) as well as intravenous glucose tolerance (117-166 III). Thus no limit could be established between a 'low' and a 'normal' early insulin response except arbitrarily. The unimodal distribution of the ER variability and the assumed high diabetic predisposition (II) do not support the hypothesis (35) that subjects with a genetically determined predisposition for diabetes would be characterized by a markedly lower early insulin response than subjects without the predisposition. This hypothesis will be further discussed in subsequent chapters.

The mean ER in the present study was 85.4 mU/l. Due to differences between the results of insulin assay from different laboratories (43) it would be hazardous to compare absolute values from the present study with that from the study. It may be mentioned however that the mean ER in men aged 50 in Uppsala, Sweden, was about 70 mU/l (85).

An analysis was performed to find out whether fasting insulin concentration, glucose tolerance test, body weight, and determinant of the magnitude of the early insulin response. Studies on insulin secretion during oral glucose tolerance test have indicated an association between fasting insulin concentration and the late phase of the insulin response. This association was found to be dependent on the body factor (8). It was then suggested that the early insulin

esponse should also be expressed as a relative change from the basal level (9). A very weak correlation was found between the fasting insulin concentration and ER in the present study. The variation in fasting insulin concentration could only explain about eight per cent of the total variance in ER. No significant correlation between these two variables was reported by Steky and Tholl (159). An error may be introduced when comparison of a fully insulin responsive subject is formed in subjects of normal weight or moderately obese subjects if the early response is expressed as per cent of fasting insulin concentration as has been done in one previous study (9, 166).

A special approach to the estimation of the magnitude of the early insulin response has been advocated by Ceras and Luft (30). They used a rapid injection of glucose followed by a constant infusion. The first sample was taken 10 minutes after the start of the glucose injection. With analogous computation of the data they judged the insulin response in relation to the blood glucose concentration assuming the $T_{1/2}$ of insulin to be 7 minutes. An insulin/glucose ratio has been used to calculate the early response in other studies also (3, 147). Inherent in this method is the assumption that the magnitude of the early insulin response is related to the degree of glucose stimulation. Studies on the relationship between concentration of glucose and insulin during a glucose tolerance test have indicated that a relationship exists between glucose stimulation and the latent phase of the insulin response (38, 41, 152). Ceras et al (38) also found a correlation between the magnitude of an intravenous glucose load and the increase of insulin concentration after ten minutes. Other studies have, however, indicated that the response relationship between glucose stimulation and early insulin response only exists at doses of glucose below five to ten grams (110, 149). At high glucose doses no further increase in early insulin response was apparent. Sumpston et al (151) reported that an increase of the glucose dose from 25 to 40 g did not increase the early response. Tholl et al (162) did not find any relationship between the glucose stimulation and early response.

In the present study the glucose dose was adjusted to body weight, which means a dose ranging from 24 to 56 grams. The glucose stimulation was administered by giving 50 g of glucose to women with diabetic body

weight and body weight (III) The glucose stimulation could only explain about one percent of the total variance in the ER variable. An error may be introduced when comparisons of daily insulin responses are performed in lean and moderately obese individuals if the early response is expressed in relation to glucose stimulation.

It is generally accepted that obese individuals have an augmented and sustained secretion of insulin after an oral glucose load indicating an association between obesity and the late phase of the insulin response as viewed by Karam et al. (103) and Kopin (105). In studies concerning the daily phases of the insulin response the association has not been evident (12, 65, 85, 97, 147, 151, 157). Usually a marked variance has been found in daily response in obese subjects indicating that some obese individuals have augmented while others have normal or subnormal response. Using group comparison tests in the often small size of patients therein a daily response was however usually not significant. Most of the women in the present study were of normal weight or only moderately obese. Only about two percent had a body weight exceeding ideal weight by 50 percent. In the total material a weak association was found between relative body weight and the early response mainly depending on a slight overrepresentation of high ER in moderately obese women. The women with low ER were only distributed in the lower relative body weight. The high variance in ER in moderately obese women compared to lean women is thus consistent with results obtained in previous studies in obese subjects.

A degree of body total glucose and blood glucose level which is a variable which in the present study is only indirectly a stepwise multiple regression analysis was performed to estimate the compound effect of the evaluable in the ER variable. The analysis indicated that the magnitude of daily insulin response was mainly determined by factors other than the glucose stimulation and the degree of obesity. These variables together could only explain about three percent of the total variance in the ER variable. The significance of the magnitude of the daily insulin response for the future development of manifest diabetes will be discussed in subsequent chapters.

SUMMARY

A weak association was found between obesity and an increased fasting insulin concentration in lean and mildly obese women aged 50 randomly selected from the general population. The variance in fasting insulin level could only be explained by the obesity variable to the extent of 10 percent indicating that tissue other than the adipose tissue is of major importance for the fasting insulin concentration.

An intravenous glucose load elicited a rapid increase in insulin concentration in almost all women with peak values at 4 minutes in the majority of cases. Different methods used to estimate the magnitude of the early insulin response may be considered comparable when the first blood sample for insulin analysis is taken within 4-6 minutes after the start of the glucose load. Error may be introduced when the response is expressed in relation to fasting insulin concentration or degree of glucose stimulation achieved after glucose loads generally used in clinical practice. A weak association was found between obesity and an augmented early insulin response.

A wide range in early insulin response was found. The frequency distribution was continuous and unimodal indicating that it is impossible to separate a group of individuals with a low response from a group with no real response except a bit slightly. The unimodal distribution of the ER also does not support the hypothesis that individuals with diabetes predominate might be characterized by a magnitude of early insulin response that markedly differs from other individuals.

Roman numeral refers to the chapter of this monograph. A letter numeral refers to the section listed pp 115-121

EARLY INSULIN RESPONSE IN RELATION TO INTRA VENOUS GLUCOSE TOLERANCE IN A POPULATION SAMPLE OF WOMEN AGED 50

Göran Blohmé

Studies on insulin secretory capacity in man as reflected by an increase in insulin concentration in peripheral blood have indicated an association between the magnitude of the early insulin response and glucose disappearance rate after an intravenous glucose load, as first observed by Samols and Marks (144) and subsequently by others (4, 22, 33, 109, 110, 115, 159). The early insulin response was suggested to be important in determining the rate of glucose decay (144). Consequently a subnormal early insulin response has been reported in subjects with a normal fasting blood glucose concentration but an impaired glucose tolerance (4, 32, 147, 151). Other studies have failed to show this pattern of insulin secretion, however (3, 94, 141). Small series of patients classified as having an impaired glucose tolerance according to different criteria and tested with different procedures might well be responsible for some of the divergent results reported.

The main purpose of this part of the present investigation was to study the association between the early insulin response and the glucose disappearance rate with special reference to women with a low glucose disappearance rate.

Material and method Chapter I

RESULTS

The variation in k value was marked; the all values of early insulin response (ER) (Fig. 1). The correlation between ER and the k value was, however, not statistically significant in the total material ($r = 0.40$, 7.91 , $p < 0.001$) as well as in women with a k value ≥ 1.00 ($r = 0.35$, 6.43 , $p < 0.001$). For both variables there was a tendency to log normal distribution (III, IV). Unlog values are somewhat high; the correlation coefficient was found in the total material ($r = 0.48$). The

att r diag am did not disclose any other mathematical fun tion between these two variables which could give a high d gree of fit. When the women w subgrouped a cording to elativ body w ight l an women had th high st value whil moderately ob wom n had th lowe t (Table I). In wom n with a elativ body w ight of 95 105 percent and a body w ght of 60 0 64 9 kg (n 47) a value of 0 47 wa found.

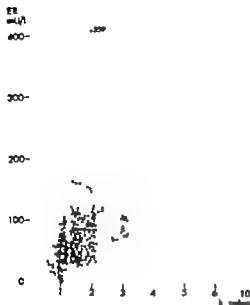


Fig 1 Relationship between k valu and ea ly insulin spon (ER) in 352 women ag d 50

Tabl L Co lation eff c nts betw n arly insulin spon (ER) and th k valu in 352 wom n subg ouped a co ding to elativ body w ght

R lativ body w ght (%)	n	
≤ 100	111	0 54
101 110	106	0 36
111 120	70	0 44
> 120	65	0 45

The correlation between ER and the k value further demonstrated in Fig 2 where women in the total material are grouped according to the deciles of ER. The k value varied markedly in all groups but did increase with increasing ER. In contrast the glucose stimulation remained almost unchanged irrespective of mode of exposure, just as the relative body weight did. Very weak correlation were frequently found between the variables G_{do} , G_{in} , G_{tim} and relative body weight, respectively, and ER as discussed in chapter IV.

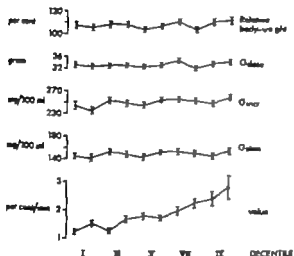


Fig 2 Relationship between relative body weight, G_{do} , G_{in} , G_{tim} and k value in 352 women grouped according to deciles of a 1 h insulin response (ER). $M \pm S.E.$

A stepwise multiple regression analysis was performed in order to turn the function of total variance in the k value, which was explained by the sum of variables ER, G_{do} , G_{in} , G_{tim} and relative body weight. The fraction was found to be 19 per cent.

Just as the variance in k value was marked at all levels of ER, the variance in ER was marked at all levels of the k value. Even in women with a k value in the lower part of the k value distribution a wide range of ER was present (Table III, Fig 3). Ten per cent of women with a k value < 1.00 had an ER below 10 mU/l, while a few had an ER even below the median ER of the total material (72 mU/l). Women with a k value < 1.00 were grouped according to relative body weight (Table IV). The mean of ER in the different groups did not deviate significantly from each other. The range in ER in the groups was wide, with the highest value in moderately obese women.

Tabl II R l i body w ight f stung neulin conc ntration and rly in ulin r pona
(ER) n 352 w m n d n wom n ubg ouped co ding to th k valu

k % /min	n	R l i w ght (%) ¹⁾	body w ght (%) ¹⁾	Fa tung in ulin (mU/l) ¹⁾	ER (mU/l) ¹⁾	Rang
0 59 9 86	352	108 7	16 5	13 9 5 2	85 4 60 4	1 559
< 0 90	21	108 6	14 2	14 9 5 8	41 3 21 4	1 88
90 0 99	18	114 1	1 8	15 5 4 4	46 8 43 2	4 169
1 00 1 09	22	115 6	23 5	14 7 6 2	55 3 26 1	8 118
1 10 1 19	22	104 6	13 0	13 7 4 9	63 6 36 9	1 169
1 20 2 29 2)	198	109 4	16 3	13 9 5 3	88 3 61 4	6 559
2 29 3 1 3)	35	104 5	14 1	13 4 5 5	99 1 51 8	28 280
≥ 3 13	36	104 8	13 2	12 9 4 3	134 0 68 8	41 376
F _{obs}		2 00		0 76	10 31	
P		> 0 05		> 0 05	< 0 01	

1) M an ± S D

2) k v lu d centile XIII 2 293

3) IX 3 126

T bl III Worm n (%) in th total m t lal bg oup d dntll of ER and a ly in uln pon (ER)
 f th tati tr al analy i worm n w th ER val bo d entll II and b l wd ntl VIII w tak n t g th

D ntl of ER

x al %/m	n	I	II	III	IV	V	VI	VII	VIII	IX
<0.90	21	33.3	9.5	28.6	14.3	9.5	4.8			
0.90-0.99	18	44.4	22.2	5.6	5.6	3.6	5.6	5.6	5.6	5.6
1.00-1.09	22	18.2	9.1	13.6	22.7	18.2	13.6		4.3	
1.10-1.19	22	4.5	18.2	31.8	4.5	9.1	4.5	13.6	4.5	4.5
1.20-2.29	198	7.1	10.1	7.6	11.6	9.6	13.1	10.6	11.1	8.6
2.29-3.12	35	5.7	5.7		11.4	8.6	5.7	17.1	8.6	14.3
>3.13	36		2.8		5.6	11.1	8.3	5.6	13.9	33.3

χ^2

86.2

P

< 0.001

Subjects
per cent

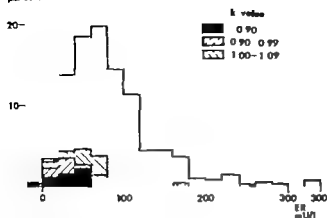


Fig 3 The distribution of w men with low k values in th percentag di tribution of th ea ly insulin r poneses (ER) in the total mat rial f 352 wom n ag d 50

Tabl IV Ea ly insulin spon (ER) in w m n with a k valu <1 00 subg ouped ac ording to r lativ body w ight

R lati b dy w ght %	n	ER mU/l		
		M an	S D	Rang
≤ 100	12	44.2	35.7	1-120
101-110	9	46.8	49.8	4-169
111-120	9	39.2	16.0	22-64
> 120	9	45.1	24.8	7-88
F _{ob}		0.08		
P		> 0.1		

Th unimodal di tribution of th ER vari ble f und in th t tal mat rial (IV) w s also pr sent when the mat rial was st ted to wom n with a k valu ≥ 1 00

The value for the second decile in the distribution of ER in the total sample was 41 mU/l. This value coincided with the mean ER of women with a k value < 0.90 . When this ER value was chosen to establish a limit between a 'low' and a 'normal' insulin response, 17 per cent of women with a k value ≥ 1.00 had a 'low' response. The mean ER \pm one S.D. for women with a k value < 0.90 was 63 mU/l. As many as 41 per cent of the total material and 35 per cent of women with k values ≥ 1.00 had an ER below this level.

Table V Some characteristics of women with an orally insulin response (ER) below 10 mU/l

Subject	Body weight kg	Relative body weight %	Fasting blood glucose mg/100 ml	k value %/min	G _{in} mg/100 ml	G _{tim} mg/100 ml
1	65.0	102	71	1.12	264	150
2	51.6	84	99	0.67	244	142
3	65.7	106	79	0.94	259	153
4	58.7	100	70	1.43	198	117
5	73.1	121	92	0.95	279	166
6	62.5	93	81	1.02	234	126
7	59.0	95	70	0.95	221	146
8	111.0	169	88	1.05	300	175
Mean	68.3	108.8	81.3	1.02	249.9	146.9
S.D.	18.3	26.6	11.0	0.21	32.6	19.1
Total material (n = 352)						
Mean	67.0	108.7	70.2	1.88	251.6	150.2
S.D.	11.2	16.5	8.3	1.10	37.9	29.1

Some characteristics of women with a very low ER (< 10 mU/l) are presented in Table V. Only one of these women had a completely normal k value. A significantly higher fasting blood glucose concentration was found in these women than in the remaining women ($t = 3.8$, $p < 0.001$) and three of them had a value exceeding the mean \pm two SD for the total material. They did not deviate from the remaining women as far as relative body weight ($t = 0.01$, $p > 0.1$) and glucose stimulation are concerned ($t = 0.1$, $p > 0.1$ and $t = 0.3$, $p > 0.1$ for G_{incr} and G_{tim} respectively).

Only one of eight women with an ER < 10 mU/l had a close familial history of diabetes (type 1) compared to 19.6 per cent in the total material. Three of seven women with children had given birth to a child with a birth weight ≥ 4.5 kg compared to 8.5 per cent in the total material. One woman had untreated hypertension and another was being treated for hypertension with chlorothalidon, which drug was withdrawn five days before the IVGTT.

DISCUSSION

A significant correlation was found between the early insulin response and the glucose disappearance rate in the present study, which is consistent with previously reported results (4, 22, 33, 109, 110, 115, 159). Although highly significant, owing to the large number of women studied, the correlation found was relatively weak. The variance in insulin response could only explain 16 per cent of the variance in k value in the total material and 12 per cent in women with a k value ≤ 1.00 . Subgrouped according to relative body weight, the explained variance was highest in lean women. A weak association between obesity and an abnormal k value (III) and between obesity and an augmented early insulin response (IV) may be considered to be responsible for the weak correlation between ER and the k value in the obese groups. In a group of women comparable as to degree of obesity and total dose of glucose, the explained variance in k value from the ER variable was 22 per cent.

The two multiple regression analyses disclosed that about 80 per cent of the variance in k value in the present study must be ascribed to factors other than early insulin response, glucose stimulation and relative body weight.

The rapid α lypha of the insulin secretion lasting for only a few minutes and the high disappearance rate of insulin from peripheral circulation, as discussed in chapter IV indicate that insulin released during the early phase has disappeared long before the period during which the glucose disappearance rate is calculated. The insulin circulating at that time thus originates from the late phase of the insulin secretion. Furthermore, the amount of insulin released during the early phase is very small. It has been estimated to account for only 2 per cent of the total extra-pancreatic insulin content in the rat in vitro (46).

The glucose disappearance rate may be assumed to depend not only on the circulating amount of insulin but also on factors determining insulin sensitivity in peripheral tissues, as discussed by Alfird et al (4). Evidence exists to suggest that the well-documented association between the early insulin response and glucose disposal is mainly indirect. Factors inherited or acquired as a consequence of the evolution of the diabetic syndrome might well partly be underlying causes of this association.

Impaired glucose tolerance

The unimodal distribution of the k value in the total material (III) indicates that a limit between normal and impaired glucose tolerance cannot be established except a bit arbitrarily. A k value below 1.00 has frequently been judged as impaired and a k value below 0.90 as markedly impaired, as discussed in chapter III. In respect of the value chosen for definition, a wide range in early insulin response was found in women with impaired glucose tolerance in the present study. The majority of the women had ER values in the low part of the ER-distribution but only a few of them as low as patients with manifest diabetes (VIII). The mean ER in the sample of women with k values below 1.00 was about half that found in the remaining women. A few women with a low k value had ER values exceeding the median for the total material, however. The wide range in early insulin response seen in women with impaired glucose tolerance is consistent with the reports (4, 85) that occurrence of an impaired glucose tolerance depends on a normal or abnormally high insulin response. This further supports the assumption that the association found between

the early insulin response and the glucose disappearance rate is mainly individual.

Low glucose disappearance rate despite a considerable concentration of circulating insulin indicates a state of insulin resistance in peripheral tissue (89). The hypoinsulinemia seen during the late phase of an oral glucose tolerance test in some patients with maturity onset diabetes is considered to be secondary to the sustained hypoglycemia and/or the often simultaneously occurring obesity as reviewed by Kipnis (105) and Porte and Bagdad (138). An increased early insulin response has been reported in a small group of subjects after marked weight gain despite a simultaneously occurring decrease in the glucose disappearance rate which indicates that the early insulin response also may be influenced by the degree of insulin sensitivity in peripheral tissue (89). The weak association between obesity and an augmented insulin response found in the total material in the present study (IV) was not, however, evident in women with $k_{it} < 1.00$. The majority of women in the present study were lean or only moderately obese. The adiposity may not be the main determinant of the degree of insulin sensitivity in peripheral tissue in the present material.

Subnormal early insulin response

Healthy individuals with a low early insulin response have been supposed to have a diabetic predisposition (35). Not generally accepted criteria exist for the definition of a low response. The unimodal distribution of the ER variable in the present population sample made it impossible to establish the limit except arbitrarily (IV).

In the study by Ceras and Luft (32) the limit between a low and a normal response was chosen arbitrarily using patients with mild manifest diabetes as unpaired intervention groups. Glucose tolerance as a function of age and weight was compared in a group of 23 of 28 diabetic patients and 85 (18 percent) healthy individuals were judged as low responders. In the present study only eight women (2 percent) had a low early insulin response as patients with mild or moderate manifest diabetes (VIII). Of these eight women only one had a completely normal k_{it} value, however. The result indicates that a low early insulin response is seen in patients with manifest diabetes very seldom compared with healthy subjects with normal glucose tolerance.

The disparate results obtained in the present study compared to the study by Cerasi and Luft (32) may be explained by difference in selection of diabetic patients and the different methods used for glucose stimulation and estimation of the magnitude of the daily insulin response as discussed in chapter IV.

A low early insulin response was supposed to characterize all stages of the diabetic syndrome (35). As a k value below 0.90 indicates markedly decreased glucose tolerance and has often been judged as diabetic (III), women with k values below this limit might be used as a reference group for the definition of a low response. As many as 20 per cent of the total material and 1 per cent of women with a k value ≥ 1.00 had an early insulin response below the mean response in women with a k value < 0.90 . Using the mean response ± 2 SD for delimitation, the corresponding frequencies in a total of 41 and 35 patients respectively.

A discussion in chapter III of a group of women with low k values may be regarded as hypothetical. Reported to it would probably delimit a group of women with a more constant decrease in the rate of glucose decay. Other women would occasionally have been classified as having pathological k values according to low degree of disturbance in the glucose homeostasis owing to methodological reasons. Moreover, it is difficult to evaluate the long-term significance of a low k value for the future development of manifest diabetes.

A prospective study might elucidate the relative significance of low glucose disappearance rate and a subnormal early insulin response for the future development of manifest diabetes.

SUMMARY

Significant correlation was found between the early insulin response and the glucose disposal rate. The ER value could only explain 16 per cent of the variance in the k value in the total material, however, indicating that there is a correlation between the two variables mainly indicated. A wide range in early insulin responses was found in women with low k values. On average, the women had a normal early insulin response. Only 10 per cent of women with a k value below 1.00 had a low early insulin response. A patient with manifest

diabetes however. The magnitude of the early insulin response was
not dependent on the degree of obesity in women with low k value.

The continuous and unimodal distribution of the ER variable found
in the total material was also found in women with a k value ≥ 1.00 .
No limit could be established between a normal and a subnormal ER
except a bit rarely. Seventeen per cent of women with a k value ≥ 1.00
had early insulin response below the mean ER for women with a k
value below 0.90.

Eight women (2 per cent) in the total material had as low early
insulin response as patient with manifest diabetes. Only one of
them had a completely normal k value. This indicates that as low
early insulin responses are seen in patients with manifest diabetes
very seldom occur in healthy middle aged women in the general popu-
lation.

Roman numeral of the chapters of the monograph. A table
numeral to the first page. 115 121

INTRAVENOUS GLUCOSE TOLERANCE AND EARLY INSULIN RESPONSE IN WOMEN AGED 50 WITH A FAMILY HISTORY OF DIABETES

Göran Blohmé

It is generally accepted that the hereditary factor plays an important role in the development of manifest diabetes. This conclusion is based on the general agreement about the mode of inheritance (7, 8, 125, 142, 155). The perspective of the mode of inheritance is also pronounced predisposition for diabetes might be expected in women with than in women without a family history of the disease. Previous studies have not indicated an association between family history of diabetes and impaired glucose tolerance, however, as evidenced by Wahlberg (166). The period before the development of impaired glucose tolerance is called the pre-diabetic period (51). It has been suggested that healthy subjects with a genetic predisposition of diabetes will be characterized by a subnormal early insulin response (15).

The main purpose of the present investigation was to study the glucose disappearance rate and the early insulin response in women aged 50 with a family history of diabetes.

For material, methods and findings see Chapter I.

RESULTS

Glucose disappearance rate

The mean value and the frequency of women with a clinically low 100 in women with diabetes, both fasting and during were not significantly low than in the control group of women without a family history of diabetes (Table I). The 50 women with a family history of diabetes in the study had lower values than the reference group. The average value was lower in women with a family history of diabetes (Fig. 1). On women reported diabetes in both parents. The mean value was 1.49. Six women reported diabetes in one parent and a sibling. The mean value in the group was 1.94 ± 0.69 (S.D.) which was not lower than in the control group.

Tabl 1 Relative body weight k value and early insulin response (ER) in 352 women subgrouped according to family history of diabetes

Family history of diabetes	n	Relative body weight (%) ¹⁾	k value (%/min) ¹⁾	k value < 1.00 %	ER (mU/l) ¹⁾	ER < 42 mU/l %
1 Mother	39	110.8	20.0	1.76 1.06	10.2	69.5 39.5 30.8
2 Father	20	110.6	13.9	1.76 0.64	10.0	80.0 52.1 25.0
3 Siblings	17	112.1	14.0	1.60 0.65	17.3	71.6 38.6 11.8
1 3	69 ²⁾	110.4	17.4	1.71 0.92	13.0	72.0 45.0 27.5
4 Others	45	106.6	16.4	1.91 0.74		82.4 46.9 22.2
5 All	239	108.6	16.8	1.90 1.20	12.6	89.9 66.5 17.6
Group 1	3	1/2	0.78	1.22	0.1	2.10 1.81
Group 5	P	> 0.1	> 0.1	> 0.1	< 0.025	< 0.05
Group 4	1/2					
Group 5	P				0.72	0.73
					> 0.1	> 0.1

1) Mean \pm SD

2) Seven women reported double heredity

Subjects
per cent

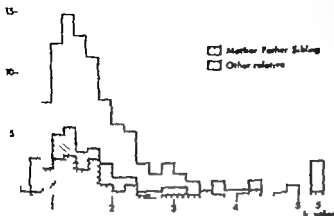


Fig 1 The distribution of women with a family history of diabetes in the percentage distribution of the k value in the total material of 352 women aged 50

Early insulin dependence

A significantly lower mean early insulin dependence (ER) and a significantly higher frequency of ER values below 42 mU/l were found in women with a close familial history of diabetes than in women without a family history of the disease (Table I). The proportion between a close familial history and a subnormal ER remained when women with a k value below 1.00 were excluded from the calculation (Table II). In this group of women with a k value ≤ 1.00 significantly higher frequency of subnormal ER values was also found in women with a family history of diabetes in both late and intermediate groups (Table II). The average ER was markedly increased in women with a family history of diabetes (Fig 2). The women with diabetes in both parents had an ER of 83 mU/l which may be compared with a mean ER of 72 mU/l in the total material. The six women who reported diabetes in one parent and a sibling had a mean ER of 77.5 ± 34.1 (SD) mU/l which was not significantly lower than in women in the intermediate group ($t = 0.45$, $p > 0.1$).

Table II Relationship between body weight, height, and early response (ER) in 313 men with normal glucose tolerance (kaju ≥ 100) subgroup according to family history of diabetes

Family history of diabetes	Relationship body weight (%)	Height (m)	k t (%/m m)	(mU/l)	ER	ER < 42 mU/l					
					Range	%					
1 Male	35	109.1	18	1.86	1.08	73.7	39.3	23	172	34.3	
1 Female	18	111.1	14.4	1.85	0.61	83.3	53.8	27	260	22.2	
1 Subnormal	14	112.1	14.8	1.76	0.61	79.8	36.6	36	150	7.1	
1 Normal	60 ²⁾	109.4	16.5	1.83	0.91	77.4	44.1	23	260	23.3	
4 Other	11	45	106.6	16.4	1.91	0.74	82.4	46.9	24	244	22.2
5	208	108.3	16.2	2.08	1.21	96.2	67.1	1	559	1.5	
Group 1	3	1/2	0.46	1.48	2.05	< 0.025	2.07				
Group 2	5	P	> 0.1	> 0.05	< 0.025	< 0.025					
Group 3	4	1/2	0.91	0.91	1.31	> 0.05	1.69				
Group 4	5	P	> 0.1	> 0.1	> 0.05	< 0.05					

1) Mean \pm SD

2) Standard error reported double

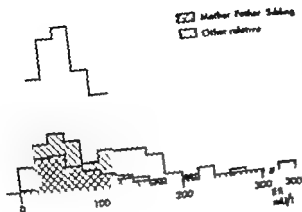


Fig 2 The distribution of women with a family history of diabetes in the percentage distribution of the daily insulin response (ER) in the total material of 352 women = aged 50

Obesity

The mean relative body weight was not significantly higher in women with a family history of diabetes than in women in the reference group (Table I II). The percentage of women with a relative body weight above 120 per cent was how high among women with a close familial history of diabetes (19.16%) than in the reference group (40 of 238) ($p = 0.025$). The mean value in the obese women with a close familial history was 153 ± 0.54 (S.D.) and in women with a close familial history was not significantly lower than in obese women in the reference group (185 ± 1.16 and 187.7 ± 8.1 ; U/I: $t = 1.12$, $p > 0.1$ and $t = 1.47$, $p > 0.05$ respectively).

Patient age

Women with patients whom on whom both had died before the age of 70 did not have significantly lower mean age of onset of diabetes a significantly higher frequency of ketonuria ($p < 0.001$) and a lower response to oral glucose tolerance test (Table III). Neither was there any difference in body weight or relative body weight between women with a ketonuria and women with a normal response to oral glucose tolerance test.

The mean age of onset of diabetes in women with a ketonuria was 10.1 (S.D.) compared to 12.7 (S.D.) in the women with a normal response to oral glucose tolerance test. The corresponding values for the women with a ketonuria and women with a normal response to oral glucose tolerance test were 68.14 and 68.14 respectively.

($t = 1.0$, $p > 0.1$) The mean age of mothers of women with an ER < 42 mU/l was 75.1 ± 9.6 (S.D.) compared to 70.5 ± 12.9 (S.D.) in the remaining women. The corresponding value for fathers were 67.8 ± 14.4 and 70.9 ± 13.8 ($t = 1.64$, $p > 0.05$).

Table III Relative body weight, k value and early insulin response (ER) in 238 women without known family history of diabetes subgrouped according to ages of the parents (at the time of study or at death)

	n	Relative body weight (%) ¹⁾	k value (%/min) ¹⁾	k value < 1.00 %	ER (mU/l) ¹⁾	ER < 42 mU/l %
Both parents not \geq 70 years of age	100	108.3 ± 7.4	1.84 ± 1.18	11.0	85.6 ± 56.5	18.0
On one both parents not \geq 70 years of age	138	109.9 ± 5.4	1.98 ± 1.22	13.8	9.3 ± 11	17.4
t/s		0.75		0.63		
■		> 0.1		0.1		

¹⁾ Mean \pm S.D.

DISCUSSION

Glucose tolerance

In the present study, no association was found between a known family history of diabetes and decreased tolerance to an intravenous glucose load. This finding may be influenced by a number of factors. The subjects were not selected with results obtained in other studies. The glucose tolerance test using intravenous (11, 126, 137, 166) as well as oral glucose load as described by Wahlberg (166).

A group of women without known family history of diabetes was used as a control group in the present study. Some women may have been accidentally forgetting family history of diabetes as none of both parents was known to have died at a young age. The prevalence of mild diabetes was low. Unknown as a of manifest diabetes

as well as subclinical forms of diabetes among relatives (discussed in chapter II) may have further contributed to a heterogeneous composition of the French group. If the reference group was restricted to women both of whose parents had reached the age of 70 no further information was gained concerning the association between a family history of diabetes and impaired glucose tolerance. However, further from the mean ages of parents of women with a k value < 1.00 was not significantly lower than in the remaining women. The results indicate that the excess of women with impaired glucose tolerance concerned fairly negatively concerning family history of diabetes owing to death of parents at early age which might have concealed an association between impaired glucose tolerance and a family history of diabetes.

The results indicate that genetic factors appeared to be involved in the evolution of the diabetic syndrome has not yet given any information on the tolerance to an intravenous glucose load in women aged 50. Although the prevalence of manifest diabetes as well as of impaired glucose tolerance will increase after the age of 50 an association might well have been found if an older age group had been studied.

Early insulin response

The prevalence of a diabetic predisposition in women (II) will probably exceed the prevalence of an impaired glucose tolerance defined as a k value < 1.00 found in women aged 50 (III). This indicates that even some women with a normal k value at the age of 50 might well have a predisposition to the disease. A low early insulin response has been suggested to be characteristic of subjects with a hereditary disposition (35).

In the present study a weak correlation was found between a clinical familial history of diabetes and a subnormal early insulin response. The correlation with the subnormal response suggested in the healthy offspring of two parents with diabetes (143, 148, 151) and in siblings of juvenile diabetics (159). A constant finding in these studies as well as in the present study was a widening in early insulin response in subjects genetically predisposed to diabetes as well as in healthy controls with a family history of the disease. Furthermore, the only woman in the present study who reported diabetes in both parents had an ER above the median value of the total material. This finding

parallel results obtained by others (32 143 151) The mean ER in women who reported diabetes in one parent and a sibling in the present study was at the same level as in women with simple heredity In addition other studies have not revealed an association between a family history of diabetes and a low early insulin response (26 33 36 114 123)

The probably heterogeneous composition of the reference group in the present study has been discussed above No further information was gained concerning the association between a family history of diabetes and subnormal early insulin response when the reference group was restricted to women with elder parents however The reference group might however conceal a considerable number of women with a genetically determined predisposition for diabetes The association found may thus have been underestimated The results indicate either that the genetic association with the diabetic predisposition may influence the initial level of the early insulin response as suggested by Cerasi and Luft (35) or that a decrease in this response has already started during the prediabetic period in some subject with a diabetic predisposition who subsequently develop manifest diabetes

A very high concordance rate with regard to diabetes has repeatedly been found in monozygotic twins as reviewed by Harvald (82) A concordance rate approaching 100 per cent was assumed Healthy members of monozygotic twins discordant regarding diabetes would thus be ideal for studies concerning a biochemical marker of the diabetic genotype Studied in a few healthy members of pairs of monozygotic twins the other member of which was diabetic has given contradictory results concerning the early insulin response however In the study of Cerasi and Luft (34) two of six healthy members of pairs of twins discordant regarding diabetes had an early insulin response slightly below and four above the limit established to disqualify from a normal response Completely normal early insulin responses have been registered more recently in other healthy monozygotic twins the other members of which had diabetes (36 49 159) A recent study in monozygotic twins has indicated as low concordance rate as 50 per cent in twins in whom diabetes developed before the age of 40 in the diabetic twin while the concordance rate was almost 100 per cent when diabetes had developed after the age of 40 (161) It was assumed that

environmental factor we of major importance for the development of diabetes in discordant twin. In the biologic studies on early insulin response in twin the diabetes developed before the age of 40 in the diabetic twin. The twins studied may thus be heterogeneous as to type and degree of genetic influence on the diabetic predisposition. Result obtained in twin studies concerning biochemical markers for the diabetic genotype must be judged with caution until further information is available on the mode(s) of inheritance of the diabetic syndrome.

An association was found between a close familial history of diabetes and obesity in the present study. It was widely held that obesity is associated with the onset of manifest diabetes (52, 101, 164, 168). The small number of obese women with a family history of diabetes and the wide range in early insulin response in these women as well as in obese women without a family history of diabetes do not permit more detailed analysis of the association between family history of diabetes, obesity and early insulin response. A prospective study may give further information about the relative significance of the factors in the evolution of the diabetic syndrome.

SUMMARY

No association was found between a family history of diabetes and glucose disappearance rate indicating that genetic factors supposed to be involved in the evolution of the diabetic syndrome may not be of importance for the development of impaired tolerance to an intravenous glucose load in women aged 50.

A weak association was found between a close familial history of diabetes and a subnormal early insulin response. The results indicate either that the genetic association with the diabetic predisposition may influence the initial level of the early insulin response or that the present study had detected as a consequence of the evolution of the diabetic syndrome.

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DIETARY HABITS AND BODY COMPOSITION IN A POPULATION SAMPLE OF WOMEN AGED 50 WITH SPECIAL REFERENCE TO DECREASED GLUCOSE TOLERANCE AND/OR SUBNORMAL EARLY INSULIN RESPONSE

Ragnhild A. and son Lenne and Göran Blohmé

Obesity often precedes the manifestation of maturity onset diabetes (5, 101, 164, 168). In addition to the excess of energy intake in relation to expenditure the qualitative composition of the food may also be a factor which together with supposed genetic factors will influence the onset of the manifestation of the diabetic syndrome (164, 168). Dietary habits in western countries with a high percentage of calories in the form of fat and refined sugar have been suggested to be responsible for the increasing prevalence of manifest diabetes (28, 40, 175). The result of the Icah Hatt Study has not confirmed the suggestion concerning the significance of the qualitative composition of the diet (102). Furthermore, it is shown that a body weight reduction has been found to improve glucose tolerance both in healthy individuals and in subjects with impaired glucose tolerance as discussed by Brunell et al (21). Andersson et al (6) and Holton et al (89).

A period of glucose intolerance by dysfunction chemical or subclinical diabetes (51) considered to precede the onset of the manifestation of the diabetic syndrome. One purpose of this part of the present investigation was to study whether women with a low glucose response to an oral or intravenous glucose load differed from randomly selected women in respect of dietary habit or body composition.

A low early insulin response has been suggested to characterize individuals with a diabetic predisposition who still have a normal glucose tolerance (35). Few data are available concerning the influence of different diets on the early insulin response. Preliminary data have indicated that weight maintenance with a high carbohydrate content was followed by an increase in the early insulin response (109) while a gradual development of dietary carbohydrate was followed by a decreased response (47). As the object of this part of the present investigation was to study whether women with a low early insulin

response with or without a 0.05-minimal IWS. The rate differed from randomly selected women in the prevalence of habits and body composition.

MATERIAL

A random sample of women aged 18 to 35 years was selected for the present study. Only women with a value ≥ 1.00 and an early insulin response ($ER \geq 4$ mU/l) were included (n = 41) and acted as a reference group. Of the 3 participating in the study (Table I). Only 26 of 30 women in the population study with an impaired glucose tolerance had a Δ value < 1.00 (III) were included (group A vs group B). Women in the population study with a Δ value ≥ 1.00 and a normal glycaemia were defined as an $ER < 4$ mU/l (IV). The ER value < 4 mU/l represented the normal ER distribution in the population study and included with them an ER in women with a Δ value < 0.90 .

Table I Number of women studied on a normal dietary history in group A, B, C and D in proportion to the total number with Δ ≥ 1.00 in the population study

k value %/min.	ER mU/l	
	< 4.2	≥ 4.2
	< 1.00	
	12/21	14/18
	Group A	Group B
≥ 1.00	27/50	36/41
	Group C	Group D

A non-participating woman was selected on non-participating women in the primary examination and the IVGTT has been performed (14 I). Body weight and relative body weight determined the time of the IVGTT of women who did not participate in the dietary history study. The present in

Chapter VII

DIETARY HABITS AND BODY COMPOSITION IN A POPULATION SAMPLE OF WOMEN AGED 50 WITH SPECIAL REFERENCE TO DECREASED GLUCOSE TOLERANCE AND/OR SUBNORMAL EARLY INSULIN RESPONSE

Ragnhild Arvidsson Lennér and Göran Blohmé

Obesity often precedes the manifestation of maturity-onset diabetes (52, 101, 164, 168). In addition to the excess of energy intake in relation to expenditure, the qualitative composition of the food may also be a factor which together with predisposed genetic factors will influence the onset of the manifestation of the diabetic syndrome (164, 168). Dietary habits in weight increase with a high percentage of calories in the form of fat and refined sugar have been suggested to be responsible for the increasing prevalence of manifest diabetes (28, 40, 175). The results of the Isala Hest Study have not confirmed the suggestion concerning the significance of the qualitative composition of the diet (102). Furthermore, short-term carbohydrate feeding has been found to improve glucose tolerance both in healthy individuals and in subjects with impaired glucose tolerance as discussed by Brunsell et al. (21). Anderson et al. (6) and Hinton et al. (89).

A period of glucose intolerance by definition chemical or subclinical diabetes (51) is considered to precede the onset of the manifestation of the diabetic syndrome. One purpose of this part of the present investigation was to study whether women with a low glucose disposal rate after an intravenous glucose load differed from a normally lipid women in respect of dietary habit or body composition.

A low early insulin response has been suggested to characterize individuals with a diabetic predisposition who still have a normal glucose tolerance (35). Few data are available concerning the influence of different diet on the early insulin response. Preliminary data have indicated that weight maintenance diet with a high carbohydrate content was followed by an increase in the early insulin response (109) while a euglycemic period of dietary carbohydrate was followed by a decrease in disposal (47). As the object of this part of the present investigation was to study whether women with a low early insulin

RESULTS

Meal frequency

The number of full meals per day varied between 1 and 3 in women in all groups with a mean of 1.5-1.8. Snacks such as coffee with bread and butter varied between 1 and 6 per day with a mean about 3 per day in all groups.

Food consumption

Food energy, fat and carbohydrate. The consumption of food energy, fat and total CHO showed a wide range in all groups (Fig. 1, 2, 3). No significant differences were noted between the groups in the means of these nutrients tested by one-way analysis of variance (Table II). About 15.5 per cent of the energy was supplied from protein in 43.5 per cent from fat and 43 per cent from CHO in the lean group.

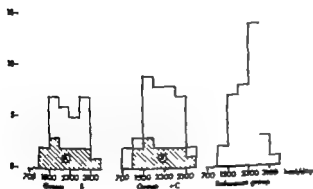


Fig. 1 The distribution of food energy/day in the total of women aged 50

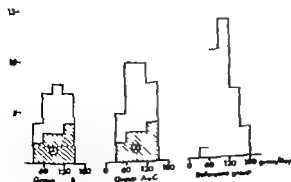


Fig. 2 The distribution of food fat/day in the total of women aged 50

Tabl IV Content of nutrients in 1000 kcal of women aged 50 n not significant.

Group	Carbohydrates									
	I		II		III		I+II+III			
	P	T	Total	Su	Lo	Other	sugar	g	g	g
A	38.8	6.3	44.4	6.0	102.15	16.6	12.7	11.9	39.11	
B	37.2	4.9	42.2	6.9	110.12	19.9	10.4	16.11	45.1	
C	36.8	6.8	43.3	6.6	105.1	19.11	11.5	11.8	41.10	
R	33.4	4.9	46.6	6	105.15	2.10	9.6	11.9	4.12	
F _{ob}	1.75	2.30	0.79	1.31	1.16	1.20	0.67			
p	< 0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.			

 1) M \pm SD

Table V Consumption of vitamins and minerals in the diet of patients with various forms of anemia

	Ca mg ¹⁾	Fe mg ¹⁾	Vitamin A IU	Thiamin mg ¹⁾	Riboflavin mg ¹⁾	Niacin mg ¹⁾	Ascorbic acid mg ¹⁾
A	1210	14	4150	1.7	2.1	13.1	9
B	1220	16	5010	1.7	0.5	14.3	104
C	110	16	4900	1.6	0.5	13.5	96
Ref. group	1040	15	4720	1.5	0.4	12.5	97
F ₂	0.98	0.97	0.57	0.28	0.70	1.18	3.35
P	n				n	n	< 0.05

1) Mean ± SD

2) Probability

A strong correlation was obtained in the total material ($n = 89$) between body fat calculated by the isotope dilution method and relative body weight calculated as a percent of ideal weight ($r = 0.92$) (Fig. 5). When computed within the groups A + B, C and R the correlation remained very strong ($r = 0.93$, 0.89 and 0.93 respectively).

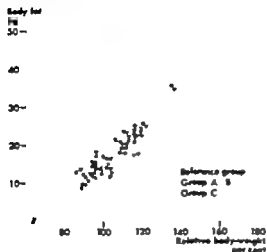


Fig. 5 Relationship between relative body weight and body fat in a material of women aged 50.

Weight reduction

Eighty-nine and 26 percent respectively of women in groups A, B and C stated that they had tried to reduce their body weight periodically, continuously during the last few years compared to 28 percent of the reference group ($\chi^2 = 2.0$, $p > 0.1$). The method used for weight reduction was numerous forms of total starvation during one or several days a week to systematic exclusion of sweet and fat from the diet. The result of the restriction was however poor as the majority of women reported unchanged or slightly increased body weight. Seventy-nine and 7 percent respectively of women in groups A, B and C reported a weight gain of more than 5 kg during the last 5 years compared to 28 percent in the reference group ($\chi^2 = 4.7$, $p > 0.1$). The vast majority of the women had a weight gain between 5 and 10 kg. Data of weight gain was also obtained for women classified as non-participating in the present study by the study for girth and difference in the frequency of women with a weight gain of more than 5 kg was found when participating and non-participating women were analysed together ($\chi^2 = 6.6$, $p > 0.05$).

DISCUSSION

Additional to the above method for the present analysis of the mean food intake during a certain period of time (48). Many errors may be introduced as has faulty memory and difficult in cooperation. The technique used in the present study was a fully worked out and information obtained was checked and double checked. Changes in body weight were discussed and personal information was collected during subgroup of the dietary carbohydrate. On a different sample specially trained dietitian performed all interview. Healthy middle aged women may be expected to cooperate well in such an interview. In spite of the favourable condition only group comparison and regression analysis were performed.

Energy supply and energy balance

The estimated mean caloric consumption was consistent with the theoretical requirement of energy in middle aged women with light to moderate activity calculated according to FAO/WHO (2010 2230 kcal/8493 MJ) (61). Another large sub sample of the present population investigated by Laksen (93) using a somewhat different technique for dietary history showed a mean caloric consumption which agreed very well with the findings of the present study. In an earlier population study of women in Gotborg Sweden (76) the mean energy intake in a group of women aged 50 was calculated to be approximately 1500 kcal. The latter study was however performed with a different method (24 hour recall) which is considered to give low values (93 173).

No significant difference existed in energy supply between the subgroups in the present investigation. However, the energy difference in body composition, namely body weight during the last five years. The ultimate difference between the body weight and the balance between energy supply and energy expenditure was an important biological condition and/or abnormality in lipid metabolism in middle aged women.

In the present study an y method was used for the calculation of the body weight. The total energy balance was calculated from a height weight table and the total energy method sufficient to meet the total energy requirement of middle aged and moderately obese women. The energy balance was calculated from a height weight table and the total energy method sufficient to meet the total energy requirement of middle aged and moderately obese women.

Qualitative composition of the diet

Reference group The dietary habit among women in the reference group has been compared with a previous report on food consumption in Sweden (171). When calculated per unit of energy the nutrient content in the food of women in the present study was about the same as in the average Swedish daily food consumption. The consumption of calories was however only about half of the mean caloric consumption calculated for the total Swedish population. This finding is consistent with the results reported by Yudkin (176) who found a low and general intake of calories. The statistics reported by Abrahamson (2) from an investigation performed in 11 European countries by Euratom also confirmed an generally reduced caloric consumption of subjects. The calculated intake of vitamins and minerals was consistent with previous reports in Sweden (171) and may be judged as sufficient according to the norms suggested by Eeg-Larsen et al (57).

In women the relationship between consumption of fat and weight (174). In the present study this relationship was weak which may be explained by the sex and age dependent differences in dietary habits. The food energy supplied was most dependent on the amount of fat consumed.

Women with mild diabetes tolerated. Women studied with less than 100 consumed slightly different food quality than the reference group with a generally higher protein concentration. The diet differed in the concentration of fat, total carbohydrates, and vitamins. The general anti-lipid diet for women with obesity body weight and probably most essential in maintaining a balanced weight gain. The thermally maladjusted subjects found between women with an impaired glucose tolerance and the reference group may not be attributable to the subjects. In addition no general correlation was obtained between the glucose tolerance and the total fat intake.

The diet of the subjects with qualitative composition of the diet in the present study showed the development of a low glucose tolerance in the subjects. The consumption of food was not related to how the subjects felt the quality and/or quantity of the food. The diet may be considered as important in the

future development of the diabetic syndrome to the manifestation of the disease. Weight gain in women with low glucose disposal rate may be suggested to be of special importance for the development of manifest diabetes.

The ordinary mean carbohydrate intake found in the dietary history investigation in the present study was 200-250 g per day and only a few women reported a carbohydrate intake below 150 g per day. A diet low in carbohydrates has been found to decrease the tolerance to glucose as discussed by Brunzell et al (21) and Anderson et al (6). The carbohydrate loading during the day before the IVGTT was considered to have masked a decreased glucose tolerance which may have been found in women on a low carbohydrate diet as exemplified during a weight reducing regimen.

Women with subnormal plasma insulin response. No significant difference was found in food quality between women with a k value ≥ 1.00 and a subnormal plasma insulin response defined as in the present chapter and women in the reference group. The result indicates that subnormal plasma insulin response is a modified symptom, does not depend on deviation from ordinary dietary habits. External diets might, however, cause changes in the plasma insulin response just as described for the relationship of the insulin response as viewed by Malm (119). Plasmalogen reports have indicated that a weight maintenance diet improved carbohydrate tolerance in a decreased plasma insulin response while carbohydrate rich diet might cause an increased response (47-109). In addition weak but significant correlations were obtained between total accumulation of energy fat and a correlation respectively and ER in the reference group in the present study. An increased plasma insulin response has been reported after a weight gain due to diet with high content of fat despite a simultaneously occurring decline in glucose tolerance (89). The increase in weight might stem from the decrease in plasma insulin sensitivity in peripheral tissue associated with the static obesity and not from the fat concentration in the adipose tissue. A significant negative correlation was found between body fat and an augmented plasma insulin response in the total material of the present population study (IV). From the study it is concluded to include the following factors and biological factors in a quantitative and qualitative change in dietary habits on the plasma insulin response.

SUMMARY

A carefully worked out technique for studying dietary history was used to investigate dietary habits in a maternal of women aged 50. The mean caloric consumption was 2260 kcal in a randomly selected group of the women. About 13.5 per cent of the energy was supplied from protein, 43 per cent from fat and 43 per cent from CHO in this group. The intake of sucrose was about half of the mean consumption calculated for the total population, probably depending on a decreasing intake of sucrose with age.

Women with ketonuria below 1.00 and/or subnormal early insulin response did not differ from the randomly selected women in respect of energy supply, body composition or recent changes in body weight. Numerically small differences in the qualitative composition of the diet with a slightly significantly high protein concentration in the diet of women with low ketonuria compared to the randomly selected women may not be attributable to an error of selection. The results indicate that the ordinary dietary habits in Sweden are not a major determinant of the development of a low glucose tolerance rate or a subnormal early insulin response in middle-aged women.

EARLY INSULIN RESPONSE IN MATURITY ONSET DIABETES MELLITUS

Göran Blohmé and Johan Waldenström

One of the main purposes of the present population study was to determine the prevalence of low early insulin response in the general population. The basis for this was the hypothesis that a low early insulin response might characterize individuals with genetically determined predisposition for diabetes (35). No accepted criterion exists by which to establish a limit between a low and a normal response. In the study by Cerasi and Luft (32) patients with mild maturity onset diabetes impaired glucose tolerance were used as a reference group. A few studies have been performed on small numbers of patients with manifest diabetes (18, 30, 95, 123, 144, 147, 151, 165). No overall low early insulin response was reported in the majority of these patients. Due to discrepancies between the results of insulin assays from different laboratories (43) data from these studies cannot be used for identification of low early insulin response in the present population study.

The main purpose of this part of the present investigation was to study the early insulin response in mildly aged patients with mild or moderate maturity-onset diabetes using the same technique of glucose stimulation and estimation of the magnitude of the early insulin response as used in the population study.

MATERIAL

Mildly aged mild or moderate maturity onset diabetes 18 women and 16 men, were selected from an outpatient clinic of diabetes in Göteborg, Sweden using criteria mentioned in chapter I. Their mean age was 52.8 and the range 40-61 years. The patients had been referred to the clinic during the previous few years for the investigation of glucose tolerance with or without clinical symptoms of manifest diabetes. The absence of weight and tiredness. The mean period between the confirmation of the diagnosis of diabetes and the study was 1.6 years with a range from 1 month to 6 years. The majority of

patient had already been advised before the first visit to the clinic to reduce their consumption of carbohydrates. During the observation at the clinic all patients had once or repeatedly had glucosuria of varying degree and a falling blood glucose concentration exceeding 120 mg/100 ml. A classification of the patients according to the initial fasting blood glucose concentration is given in Table I.

Table I Initial fasting blood glucose concentration in 34 diabetic patients

mg/100 ml	n
< 120	1
120-149	5
150-199	14
≥ 200	14

The nine patients with initial fasting blood glucose concentration of 120 mg/100 ml or below 120 mg/100 ml after a period of excellent weight compensation from the diet followed by decreasing glucose and weight loss of 4 kg were subsequently found to have a fasting blood glucose level at or below 140 mg/100 ml. One of these patients had been treated with insulin. Eight patients continued with the diet and one also began to take a few weeks. The therapy was interrupted in two patients 3 months and patient 10 6 months before the study. Since the first visit all patients had been placed on a diet and a diary diet for a period of 12 months of all meals and carbohydrates. A weight loss of 16.4 ± 4.9 kg (range 0-18 kg) occurred in the first 6 months. The highest daily intake of carbohydrates was 100 g. The treated mean body weight in the 3 years before the diet was 84.4 ± 17 kg (range 61-136 kg). The actual weight was 68.0 ± 14.1 kg (range 57-111 kg) and relative body fat was 60% (range 40-81%). Diet weight and weight loss were the top order of 150 g/day. The body fat and fasting blood glucose concentration decreased of the 3 years of the diet. A few patients with newly

detected disease still had a marked fasting hypoglycemia at the time of the present study (see results). The diabetic patient reported a family history of diabetes in 68 percent (Table II) according to criteria in chapter I.

Table II Prevalence of known family history of diabetes in 34 diabetic patients

	n	%
Close familial history	17*	50.0
Mother	10	29.4
Father	7	20.6
Sibling	5	14.7
Other relative	6	17.6

* For patient reported family history in more than one of the subgroup

Two patients (cases A and B) having markedly increasing weight will be described in more detail.

Case A is a woman aged 57. Her mother and maternal aunt had maturity onset diabetes. She had not suffered from liver or gall bladder or pancreatic disease. She had been treated for hypothyroidism with salutaridin in the early 1960s. Clinical weight gain was noted by the Tetratap method a few weeks of this in March 1968. A few days later she was found to be thin. Her fasting blood glucose concentration was 160 mg/100 ml and her postprandial blood glucose by the Clin-tex method. An oral glucose tolerance test was performed (50 g of glucose orally spaced at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 255, 270, 285, 300, 315, 330, 345, 360, 375, 390, 405, 420, 435, 450, 465, 480, 495, 510, 525, 540, 555, 570, 585, 600, 615, 630, 645, 660, 675, 690, 705, 720, 735, 750, 765, 780, 795, 810, 825, 840, 855, 870, 885, 900, 915, 930, 945, 960, 975, 990, 1005, 1020, 1035, 1050, 1065, 1080, 1095, 1110, 1125, 1140, 1155, 1170, 1185, 1200, 1215, 1230, 1245, 1260, 1275, 1290, 1305, 1320, 1335, 1350, 1365, 1380, 1395, 1410, 1425, 1440, 1455, 1470, 1485, 1500, 1515, 1530, 1545, 1560, 1575, 1590, 1605, 1620, 1635, 1650, 1665, 1680, 1695, 1710, 1725, 1740, 1755, 1770, 1785, 1800, 1815, 1830, 1845, 1860, 1875, 1890, 1905, 1920, 1935, 1950, 1965, 1980, 1995, 2010, 2025, 2040, 2055, 2070, 2085, 2100, 2115, 2130, 2145, 2160, 2175, 2190, 2205, 2220, 2235, 2250, 2265, 2280, 2295, 2310, 2325, 2340, 2355, 2370, 2385, 2400, 2415, 2430, 2445, 2460, 2475, 2490, 2505, 2520, 2535, 2550, 2565, 2580, 2595, 2610, 2625, 2640, 2655, 2670, 2685, 2700, 2715, 2730, 2745, 2760, 2775, 2790, 2805, 2820, 2835, 2850, 2865, 2880, 2895, 2910, 2925, 2940, 2955, 2970, 2985, 3000, 3015, 3030, 3045, 3060, 3075, 3090, 3105, 3120, 3135, 3150, 3165, 3180, 3195, 3210, 3225, 3240, 3255, 3270, 3285, 3300, 3315, 3330, 3345, 3360, 3375, 3390, 3405, 3420, 3435, 3450, 3465, 3480, 3495, 3510, 3525, 3540, 3555, 3570, 3585, 3600, 3615, 3630, 3645, 3660, 3675, 3690, 3705, 3720, 3735, 3750, 3765, 3780, 3795, 3810, 3825, 3840, 3855, 3870, 3885, 3900, 3915, 3930, 3945, 3960, 3975, 3990, 4005, 4020, 4035, 4050, 4065, 4080, 4095, 4110, 4125, 4140, 4155, 4170, 4185, 4200, 4215, 4230, 4245, 4260, 4275, 4290, 4305, 4320, 4335, 4350, 4365, 4380, 4395, 4410, 4425, 4440, 4455, 4470, 4485, 4500, 4515, 4530, 4545, 4560, 4575, 4590, 4605, 4620, 4635, 4650, 4665, 4680, 4695, 4710, 4725, 4740, 4755, 4770, 4785, 4800, 4815, 4830, 4845, 4860, 4875, 4890, 4905, 4920, 4935, 4950, 4965, 4980, 4995, 5010, 5025, 5040, 5055, 5070, 5085, 5100, 5115, 5130, 5145, 5160, 5175, 5190, 5205, 5220, 5235, 5250, 5265, 5280, 5295, 5310, 5325, 5340, 5355, 5370, 5385, 5400, 5415, 5430, 5445, 5460, 5475, 5490, 5505, 5520, 5535, 5550, 5565, 5580, 5595, 5610, 5625, 5640, 5655, 5670, 5685, 5700, 5715, 5730, 5745, 5760, 5775, 5790, 5805, 5820, 5835, 5850, 5865, 5880, 5895, 5910, 5925, 5940, 5955, 5970, 5985, 6000, 6015, 6030, 6045, 6060, 6075, 6090, 6105, 6120, 6135, 6150, 6165, 6180, 6195, 6210, 6225, 6240, 6255, 6270, 6285, 6300, 6315, 6330, 6345, 6360, 6375, 6390, 6405, 6420, 6435, 6450, 6465, 6480, 6495, 6510, 6525, 6540, 6555, 6570, 6585, 6600, 6615, 6630, 6645, 6660, 6675, 6690, 6705, 6720, 6735, 6750, 6765, 6780, 6795, 6810, 6825, 6840, 6855, 6870, 6885, 6900, 6915, 6930, 6945, 6960, 6975, 6990, 7005, 7020, 7035, 7050, 7065, 7080, 7095, 7110, 7125, 7140, 7155, 7170, 7185, 7200, 7215, 7230, 7245, 7260, 7275, 7290, 7305, 7320, 7335, 7350, 7365, 7380, 7395, 7410, 7425, 7440, 7455, 7470, 7485, 7500, 7515, 7530, 7545, 7560, 7575, 7590, 7605, 7620, 7635, 7650, 7665, 7680, 7695, 7710, 7725, 7740, 7755, 7770, 7785, 7800, 7815, 7830, 7845, 7860, 7875, 7890, 7905, 7920, 7935, 7950, 7965, 7980, 7995, 8010, 8025, 8040, 8055, 8070, 8085, 8100, 8115, 8130, 8145, 8160, 8175, 8190, 8205, 8220, 8235, 8250, 8265, 8280, 8295, 8310, 8325, 8340, 8355, 8370, 8385, 8400, 8415, 8430, 8445, 8460, 8475, 8490, 8505, 8520, 8535, 8550, 8565, 8580, 8595, 8610, 8625, 8640, 8655, 8670, 8685, 8700, 8715, 8730, 8745, 8760, 8775, 8790, 8805, 8820, 8835, 8850, 8865, 8880, 8895, 8910, 8925, 8940, 8955, 8970, 8985, 9000, 9015, 9030, 9045, 9060, 9075, 9090, 9105, 9120, 9135, 9150, 9165, 9180, 9195, 9210, 9225, 9240, 9255, 9270, 9285, 9300, 9315, 9330, 9345, 9360, 9375, 9390, 9405, 9420, 9435, 9450, 9465, 9480, 9495, 9510, 9525, 9540, 9555, 9570, 9585, 9600, 9615, 9630, 9645, 9660, 9675, 9690, 9705, 9720, 9735, 9750, 9765, 9780, 9795, 9810, 9825, 9840, 9855, 9870, 9885, 9900, 9915, 9930, 9945, 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22830, 22845, 22860, 22875, 22890, 22905, 22920, 22935, 22950, 22965, 22980, 22995, 23010, 23025, 23040, 23055, 23070, 23085, 23100, 23115, 23130, 23145, 23160, 23175, 23190, 23205, 23220, 23235, 23250, 23265, 23280, 23295, 23310, 23325, 23340, 23355, 23370, 23385, 23400, 23415, 23430, 23445, 23460, 23475, 23490, 23505, 23520, 23535, 23550, 23565, 23580, 23595, 23610, 23625, 23640, 23655, 23670, 23685, 23700, 23715, 23730, 23745, 23760, 23775, 23790, 23805, 23820, 23835, 23850, 23865, 23880, 23895, 23910, 23925, 23940, 23955, 23970, 23985, 24000, 24015, 24030, 24045, 24060, 24075, 24090, 24105, 24120, 24135, 24150, 24165, 24180, 24195, 24210, 24225, 24240, 24255, 24270, 24285, 24300, 24315, 24330, 24345, 24360, 24375, 24390, 24405, 24420, 24435, 24450, 24465, 24480, 24495, 24510, 24525, 24540, 24555, 24570, 24585, 24600, 24615, 24630, 24645, 24660, 24675, 24690, 24705, 24720, 24735, 24750, 24765, 24780, 24795, 24810, 24825, 24840, 24855, 24870, 24885, 24900, 24915, 24930, 24945, 24960, 24975, 24990, 25005, 25020, 25035, 25050, 25065, 25080, 25095, 25110, 25125, 25140, 25155, 25170, 25185, 25200, 25215, 25230, 25245, 25260, 25275, 25290, 25305, 25320, 25335, 25350, 25365, 25380, 25395, 25410, 25425, 25440, 25455, 25470, 25485, 25500, 25515, 25530, 25545, 25560, 25575, 25590, 25605, 25620, 25635, 25650, 25665, 25680, 25695, 25710, 25725, 25740, 25755, 25770, 25785, 25800, 25815, 25830, 25845, 25860, 25875, 25890, 25905, 25920, 25935, 25950, 25965, 25980, 25995, 26010, 26025, 26040, 26055, 26070, 26085, 26100, 26115, 26130, 26145, 26160, 26175, 26190, 26205, 26220, 26235, 26250, 26265, 26280, 26295, 26310, 26325, 26340, 26355, 26370, 26385, 26400, 26415, 26430, 26445, 26460, 26475, 26490, 26505, 26520, 26535, 26550, 26565, 26580, 26595, 26610, 26625, 26640, 26655, 26670, 26685, 26700, 26715, 26730, 26745, 26760, 26775, 26790, 26805, 2

has since the initial period of fasting hypoglycemia been normal and no glucosuria has been noted. At the present study her fasting venous blood glucose concentration was 86 mg/100 ml and her HbA_{1c} value 1.55. An oral glucose tolerance test performed as above 4 months after the intravenous glucose tolerance test showed blood glucose concentrations 100, 167, 185 mg/100 ml at 0, 30 and 60 minutes respectively.

Case B is a man aged 46. He reported no family history of diabetes. He had not suffered from liver, gall bladder or pancreatic disease. Glucosuria was found at a health control in December 1970. He had no symptoms of diabetes. An oral glucose tolerance test was performed (see Table A) showing blood glucose concentrations of 90, 205, 240 mg/100 ml at 0, 30 and 60 minutes respectively. He was then referred to the clinic and one month later he had a fasting blood glucose concentration of 128 mg/100 ml and the urine contained 11 g glucose over 24 hours. He was advised to reduce his consumption of fats and carbohydrates. No drug was used. A reduction of 12 kg in his body weight was noted during half a year. He maintained this low weight and at the time of the present study his body weight was 84 kg corresponding to a total body weight of 112 per cent. Except for once (6 g/4 hours) he has been free from glucose since the first admission. At the present study his fasting venous blood glucose concentration was 73 mg/100 ml and his HbA_{1c} value 0.97. An oral glucose tolerance test was performed as above four months after the intravenous glucose tolerance test showed blood glucose concentrations of 84, 166 and 198 mg/100 ml respectively.

For methods see Appendix I.

RESULTS

The mean fasting blood glucose concentration was 71.128 mg/100 ml. The mean HbA_{1c} was 0.74 ± 0.28 (S.D.) and the range 1.55-0.1 per cent. In Table I (Fig. 1) Two patients out of whom Case B had a clinical weight markedly reduced the other patient. Two other patients had a weight of 100 and 105 per cent. Fasting hypoglycemia was associated with a low HbA_{1c} ($P < 0.01$).

k value

15

10

05

*

100

150

200

250

300

350

Fasting blood glucose
mg/100 ml

Fig 1 Relationship
between fasting blood glucose
concentration and k value
in 34 patients with diabetes

The fasting serum insulin concentration ranged from 8 to 40 mU/l with mean 16.4 ± 6.4 (S.E.) mU/l. A correlation was found between relative body weight and fasting insulin concentration ($r = 0.53$, 34 patients, $p < 0.001$). The increase of insulin concentration during the first 15 minutes after the start of the glucose load was very low or unmeasurable in all but two of the patients (Cases A and B) (Table III) irrespective of the fasting insulin level (Fig 2).

Table III Concentration of serum insulin and blood glucose when fasting and after an intravenous glucose load in 34 diabetic patients. ER = orally inulin plasma

	Diabetic patient n = 32 ¹⁾		C		A		C		B	
	Insulin mU/l ²⁾	Blood glucose mg/100 ml ²⁾	Insulin mU/l	Blood glucose mg/100 ml	Insulin mU/l	Blood glucose mg/100 ml	Insulin mU/l	Blood glucose mg/100 ml	Insulin mU/l	Blood glucose mg/100 ml
0 min.	16.2	65	164	71	25	86	9	73		
4 min.	17.6	74	443	95	61	369	88	444		
6 min.	15.1	58	415	87	48	332	69	371		
8 min.	14.6	54	394	77	47	322	41	312		
ER	16.5	3			40		69			

1) Cases A and C. B = counted for patient

2) Mean \pm S.D.

DISCUSSION

The manifest form of the diabetic syndrome over a wide range of disturbance in the glucose homeostasis. Most patients selected for the present study present a very mild form of the disease. Owing to dietary restriction and subsequent reduction in body weight and in a few cases short periods of sulfonylurea therapy, some of the patients studied had blood glucose levels comparable to normal. Fasting blood glucose concentration in two cases also was found to be normal and glucose intolerance tests were not performed.

A diet with a high carbohydrate content was prescribed during the day before the test. Carbohydrate feeding has been shown to improve the tolerance to glucose in subjects with impaired glucose tolerance and was used in patient III. A high serum alkali value and a high frequency of alkalosis of 0.90-1.00 was found than expected in other tested nondiabetic patients as observed by Wahleberg (166). The carbohydrate feeding may have improved the glucose tolerance in some of the patients though presently. The titration of mild cases will show probably the main use of the discrepancy.

In ul t en

The total glucose load is a measure in a insulin tolerance test, half of the patient's weight. The patient was 150 pounds but two pounds. The patient had 14 mg of glucose in the urine obtained from the patient's urine, 100 mg per 100 ml (Chapter IV). The difference between the patient's blood sugar and normal is 100 mg per 100 ml.

The patient's comorbidity
 with diabetes mellitus
 and hyperlipidemia
 was not a factor in the
 development of the
 acute myocardial infarction.
 The patient's hyperlipidemia
 was not a factor in the
 development of the acute
 myocardial infarction.
 The patient's hyperlipidemia
 was not a factor in the
 development of the acute
 myocardial infarction.

The ultimate in the present study a constant with result from other studies on maturity onset diabetes showing a pronounced hyporesponsivity of insulin to an intravenous glucose load especially in subjects with fasting hypoglycemia (18 50 95 123 144 147 151 165) Studies in patients with juvenile diabetes have shown an extremely low concentration of insulin in the plasma (68 96 165) as well as a latent (10 59 96 136) insulin responsiveness in the third of the population with the insulin concentration capacity

The insulin concentration during an oral glucose tolerance test is related to origin mainly from the late phase of the insulin secretion. An increase and sustained insulin response after an oral glucose load was reported in the early 1960s in maturity onset diabetes temporally ranging from the old onset form of insulin deficiency as the major defect in maturity onset diabetes (172) The hypoglycemia has however mainly been attributed to the frequent simultaneously occurring late obesity (105) An overview of the subjects was found in the diabetic group in the present study 38 percent having a relative body weight exceeding 120 percent of ideal weight compared to 18 percent in the population sample. The association between obesity and an increased fasting insulin level is found in the diabetic population consistent with the ultimate in non diabetic subjects (IV)

Significant correlations were found between relative body weight and ER in the group of patients with gestational diabetes of disturbed glucose homeostasis. The two patients with positive values of ER despite fasting blood glucose concentrations slightly in excess of 120 mg/100 ml were however below the population study value. The correlation between obesity and an augmented insulin response (IV) The result indicates that the degree of hypoglycemia is the main denominator in the magnitude of the early insulin response in subjects with maturity onset diabetes

Two patients in the present study had a quantitatively differently related insulin response with an ER of 40 and 69 mU/l respectively, values which are in the normal range and in the normal range of the ER distribution in the population study (IV) They had normal fasting blood glucose concentrations and ketone levels of 1.45 and 0.97 percent respectively. Both had mild postprandial hyperglycemia related to the insulin with glucose and moderate fasting hypoglycemia. A low ideal body weight education helped to reduce the insulin concentration in the

glucose homeostasis. It may well be that the early insulin response would have been markedly lower if measured during the fasting hyperglycemic period as none of the patients in the present study with fasting hypoglycemia had an evident increase in the insulin concentration. A slight reduction of the same magnitude or even greater was not followed by normalization of ER in other subjects in the present study despite normalization of the fasting blood glucose concentration.

A marked increase in the insulin response after an oral glucose load was reported in obese diabetics after a considerable weight reduction associated with normalization of fasting blood glucose concentration and glucose tolerance (54). In two patients with juvenile diabetes studied with oral glucose loads during a period of remission and delayed but augmented response has been reported (87-98). In another patient the magnitude of the insulin response to an oral glucose load changed inversely with the blood glucose concentration during the course of subsequent exacerbation and improvement (108). A low insulin response to an oral glucose load has been reported during periods of remission in a few other patients with juvenile diabetes (10-70-98). These results indicate that a remission in impaired glucose homeostasis may be followed by an improvement in the late insulin response. The result of the present study indicates that this improvement may also be valid for the early insulin response. An improvement in a late insulin response has furthermore been reported in a patient in remission from diabetic ketoacidosis when tested with the provocative injections of glucagon and tolbutamide (67).

Further studies on the insulin secretory capacity are needed during the post-diabetic period of remission of the glucose homeostasis to elucidate the potentially reversible nature of beta cell dysfunction.

SUMMARY

Middle-aged mild to moderate maturity-onset diabetics were characterized by a relatively low or non-measurable early insulin response ($ER < 10 \text{ mU/l}$). The patients were more pronounced in patient with fasting hyperglycemia. Two patients in remission or partial remission showed a changing glucose homeostasis after a marked weight reduction followed by a prompt decrease in insulin concentration to the level achieved with an early insulin response (ER) within the normal range as judged from the population study. The

sult indicate that an improvement may occur in the early insulin
response in some patients with diabetes during a period of remission

Roman numeral of the chapter of the monograph. Arabic
numeral of the figure, pp 115-121

GENERAL DISCUSSION

The cross sectional study of the present population sample of women aged 50 is intended to be the first phase of a long term prospective study with the main purpose of evaluating whether a subnormal early insulin response will predict the subsequent development of manifest diabetes.

The age strata of 50 was chosen as a starting point for the present study as the probability of developing manifest diabetes in Sweden has been reported to be still rather low up to this age (72). Hence for ages below 50 the age annual morbidity risk was estimated to be 0.4 per thousand while after 50 a markedly increased risk was found with age especially in women. These results are consistent with the data collected by the National Center for Health Statistics in the U.S. (52).

The prevalence of manifest diabetes (1.3 per cent) found in the present study (11) is in agreement with the general finding that 1-2 per cent of the total population in western countries has this stage of the disease (52, 62, 72). The well documented strong gender dependence in the prevalence of manifest diabetes means however that the average prevalence in the general population does not reflect the total morbidity risk for this type of the disease. The total morbidity risk may be defined as the risk to an individual of developing manifest diabetes up to the age of 90. However many of these presumptive diabetics will not develop the disease as they will die so that on account of the disease. A study of the diabetes in Sweden indicated a total morbidity risk of 1.3 per cent in women and 0.5 per cent in men (7). In the present study corresponding assumptions of probable gaps in the age distribution and sex mortality among such persons. The high prevalence figure agreed very well with the data collected by the National Center for Health Statistics in the U.S. (52). The women in the present study possessed diabetes in 1.4 per cent of their mothers and 4.9 per cent of their fathers (11). When only parents who had had diabetes up to the age of 75 were taken into account the corresponding figures were still high, 13.5 and 7.0 per cent respectively. Reasonable doubt is to be suggested however that the actual prevalence of diabetes is probably even higher (11). A population of 20 per cent has been suggested (13, 15). In the present and many samples of women aged 50 on the basis of the present study women might thus be predicted to have

a diabetic predisposition and consequently to be candidate for treatment of manifest diabetes

Population studies have indicated that an impaired tolerance to an oral glucose load is not uncommon in the general population especially in the elderly (25-52-83). The few long term follow up studies concerning the significance of an impaired glucose tolerance in the development of the manifest form of the disease (132-133-170). Available data indicate however that the rate of development of manifest diabetes in the future is related to the initial degree of impaired glucose tolerance. Subjects with borderline values may have unchanged glucose tolerance for a considerable period of time (29-133) or even show an improvement (131-133).

Available data concerning the long term significance of an impaired tolerance to an intravenous glucose load are limited to a few series of myocardial infarction followed up for up to 5 years (135-166). All who developed manifest diabetes had initially low k values.

Not reported at the present time in the present population sample as the women had taken part in several previous studies and furthermore studies are planned for the future. It is well known that the glucose disappearance rate may vary from time to time in the same individual as discussed in chapter III. A standardised test procedure including pre-treatment with a carbohydrate rich diet would in the present study in order to standardise the experimental condition is far possible.

The distribution of the k values was continuous and unimodal in the present cross sectional study which is consistent with the reports (11-116-117-166). The maximal impairment to establish a limit between decreased and normal glucose tolerance except but only Six percent of the women had a k value < 0.90 (I) present a k value < 1.00 and 17 percent a k value < 1.10 (III). Thus indicating that the distribution of the k values is a pathological part of women aged 50 years for a pathological k value is usually accepted as representing a chemical subclinical form of the diabetes syndrome. Compensable even tolerance figures have been reported in randomly selected men aged 50 in Uppsala Sweden (85). It is reasonable to assume that women in the present study with markedly impaired glucose tolerance are candidates for manifest diabetes in the future.

The weight gain in the subjects found in the present study was not due to the action in the diet of obesity or glucose stimulation (III). A correlation was found however between obesity and a decreased glucose tolerance rate which is in agreement with other reports (1185-1186-1187-1188). When studying women with a weight gain < 100 more or less no significant difference was found in body composition compared to a randomly selected reference group however (VII). This result indicates that obesity will not be the major determinant for the development of impaired tolerance to glucose injected intravenously in middle aged women. As obesity often precedes the onset of manifest diabetes (5-101-104-108) weight gain may be considered to be a major determinant for the future development of manifest diabetes.

Diabetes has been equally been considered important for the development of diabetes (8-40-175). The findings in chapter VIII do not indicate however that diabetes is a more important factor for the development of impaired glucose tolerance in middle aged women. Diabetes might together with obesity be of considerable importance to the future development of manifest diabetes as well.

An additional but one of blood glucose level after a glucose load has often been indicated as a prognostic factor for the inheritance of diabetes. Stenbom (155) and Stenbom et al (156) have discussed the hypothesis that the development of diabetes is a continuous process with no marked nodal points in the early and late stages of a multigenic disease. The continuous arterial diastolic blood pressure is a continuous variable. Despite an assumed high diastolic blood pressure in the present population sample (II) the distribution of the values was unimodal and unimodal which corresponds with the hypothesis (1186-1187-188). The influence of age on the development of diabetes is a subject of controversy. The development of diabetes with the usual predicted position on the development of a low weight gain is not as important as it is. The present study found however a family history of diabetes and a low weight gain (VI). The result on the development of diabetes (1186-1187-188). The results indicate that the subjects with a weight gain < 100 more or less no significant difference was found in body composition compared to a randomly selected reference group however (VII). This result indicates that obesity will not be the major determinant for the development of impaired tolerance to glucose injected intravenously in middle aged women. As obesity often precedes the onset of manifest diabetes (5-101-104-108) weight gain may be considered to be a major determinant for the future development of manifest diabetes as well.

In peripheral tissues might be of more importance. The genetic influence might contribute more strongly to the further development of the diabetic syndrome.

The magnitude of the daily insulin response to an intravenous glucose load as reflected by an increase in insulin concentration in peripheral blood is a biological variable regarded to be of special importance in judging the susceptibility to diabetes (35). The hypothesis that a low early insulin response is characteristic of all stages of the diabetic syndrome including the prediabetic state (34) has however been questioned as normal daily insulin responses have been reported even in patients with glucose intolerance (3, 94, 141) and in the healthy member of pairs of monozygotic twins the other member of which was diabetic (36, 49, 159). Furthermore Sumpson et al (151) have described cases in which manifest diabetes developed twenty months after moderately high early insulin response had been recorded.

The endogenous age-dependent decrease in the daily insulin response is discussed in chapter IV. The effects of insulin in response to an intravenous glucose load is considered to be almost instantaneous and the insulin concentration will decrease rapidly after the first rapid phase. Furthermore the disappearance of insulin from the peripheral blood will be rather high with a half life of insulin of 3-7 minutes (30, 153, 160, 162, 163). Data indicate that blood samples for insulin analysis must be taken very soon after the glucose load with the first blood sample being taken not later than 4-6 minutes after the start of the glucose injection. Of several methods judged to be comparable for the estimation of the daily insulin response (IV) the method adopted by Thoenes et al (162) was used in the present study. The method is based on repeated analysis of the insulin concentration during the first few minutes after the glucose load. The calculated variability of the early insulin response (ER) suggested to represent the increase in insulin concentration that would have occurred if all insulin released into the general circulation had remained within the plasma pool (162).

The insulin response was related by age and adjusted to body weight in the present study. Despite the wide range in blood glucose concentration was found during the first few minutes of the test (III). This was also evident in the relative increase of relative body weight. This will in most homogeneous groups of women a regular body weight and stable body weight. The results indicate that a wide range in the

olum of distribution of glucos and agr e with pr vious r ports
 (32 9) o signficant corr lation was found b tw n the variab
 l a G_{tm} and ER and a v ry w ak but signficant corr lation b t
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The hypothesis that a subnormal early insulin response will have act
tix all stages of the diabetic syndrome (35) means that subjects with
a subclinical form of the disease might be missed as a fasting group
to establish a limit between a 'low' and a normal response. Subjects
with a normal fasting blood glucose concentration and a k value below
0.90 are usually considered to have a subclinical form of diabetes (III).
In the present study women with a k value below 0.90 had an average
a lower ER than other women (V). Although the range of early insulin
response was considerable in the women the majority of the subnormal
ER value within the lower part of the ER distribution. This is con-
sistent with other reports (4, 85). When women with a k value below
0.90 were used as a fasting group to define a low early insulin
response a considerable part of the total population was judged a low
response. However it is obvious that groups of women with low
k values is heterogeneous regarding the insulin secretion pattern.

An association has previously been reported between the early insulin
response and the glucose disposal rate (4, 22, 33, 109,
110, 115, 144, 159, V). A discussion in chapter V thus relates
essentially to body fat. Unduly low fat may be regarded to
influence both the early insulin response and the glucose disposal rate.
The main direction of the influence on the variable will be
toward low values in subjects who subsequently develop manifest dia-
betes. An increased early insulin response has recently been reported
after a weight gain associated with diabetes in glucose disposal and
ER (89). In the present study weak but significant relations were
found between initial body weight and early insulin response in the
total material (IV) and between assumption of alcohol and glucose
on the one hand and early insulin response on the other in a sub-sample
(VII). Obesity and especially recent weight gain often precede the
onset of manifest diabetes (52, 101, 164, 168). It is evident that a low
glucose disposal rate might be accompanied for a long time by
ER rises by a normal early insulin response. The significance of the
ER level in women with low k values for the future development of
manifest diabetes needs prospective study to be determined.

The ER value had a unimodal distribution in the present study
(IV) while in other reports (85, 159) a bimodal distribution in
connection with glucose tolerance tests a unimodal distribution does not
necessarily prove multiple inheritance of a continuous variable.

GENERAL SUMMARY

The present study was performed on women aged 50 randomly selected from the female population of this age in Göteborg Sweden. The study was supported by an investigation of middle aged patients with maturity onset diabetes.

The prevalence of manifest diabetes in women aged 50 was 13 per cent which is considerably high prevalence was found in their parents. The prevalence of known manifest diabetes in the mother was 14 per cent and in the fathers 9 per cent. When only patients who had children or passed the age of 75 were taken into account the figures were still higher 15.5 and 7.0 per cent respectively. Probable underreport of diabetes among parents and the fact that a considerable part of them were living at the time of the study and thus still at risk of developing the disease indicate that the diabetic predisposition may be very high in the population studied.

The tolerance to glucose injected intravenously expressed as a peak value representing the glucose disappearance at 10 minutes prior to the test showed a wide range in women aged 50 with a mean \pm S.D. of 1.88 ± 1.10 and median value of 1.59. The distribution of the peak values was continuous and unimodal. Six per cent of the women had a peak value < 0.90 11 per cent peak value < 1.00 and 17 per cent peak value < 1.10 . A weak negative correlation was found between relative body weight and the peak value. The studying women with a peak value < 1.00 more closely than the others showed how that neither obesity nor diabetes was important factors in the development of a low peak value. No association was found between family history of diabetes and a low peak value indicating that the diabetic is thought to be associated with the diabetic predisposition and not to be a major determinant of the development of a low peak value in middle aged women.

The tolerance to glucose load is determined as of insulin administered by an insulin concentration in the peripheral blood. The limit of normal with a peak concentration with ± 4.6 mU/l. The maximum of the insulin response (ER) was not related to the blood glucose concentration. The added glucose of a few minutes of the test. The distribution of the ER values was not unimodal. Consequently, the relationship between a low and normal

pon x pt a bit a ily A significant cor lati n was found b tw n ER and th k valu Th ER va iabl xpl ined 16 p c nt of th vari an m th E valu A w ak associati on was found betw n ob sity and an augment d ER. W m m with a subnormal ER did not diff r from an domly s lected women in p e t of body mpo iti n o di ta y habit Worn n with a lo famlial hi to y of di bet had ligh but igni fl antly l w ER than worn n without.

P ti nts with maturi ty ne t d abets h d very low or non m su bl arly insulin pon Th s wa p ally vid nt in p tient with fa ting hypergly emi Two p tient in mi lion of th d turbed glu homeo tas had an bvion s ly in ulin p n aft a p od of w ight educati on. Thi ind at that a emi ion h d al m cu ed in th insulin ec ti on p acity Eight women (2 p ent) in th popu lati m ample had a l w ER a th maj i ty of the diabeti p t nt Only one of them had a ompl t ly normal k valu A low a ly insulin pon a ar en in diabeti p ti nt will c n eq ntly v ry s idom be f und in healthy muddl g d women and annot b a h a t i tic featur so th m j rity f women with a diabeti p ed sp iti n Women with a k valu < 0.90 had on v g low ER than oth women but th ang in ER was wide nd a n d abl ov l pping xted b tw n worn n with l w and worn n with n rmal k valu

In onclusi n, th finding of th pr ent inv tig ti n supp t th hypo th t th ly in ulin pons will d a d ing th ev l ti n of th d ab ts ynd om Th initial m gnitud of th ly insulin pon m y beg n ti ally d t rmined a w ll Subj t with a ubn mal a ly nsul n pon may c n q ntly on titut a h t og n g up Som subj ct m y h v a subnormal pon n quen of th evolution of th di b ts ynd om wh oth m y hav a g m ticall y d t rmin d bnormal spon n t p c ted with di bet Al g t m p osp ll tudy might d t rmin th l ti signif can off to ch low k al a bnormal ly i sul n pon obes ty d etary h bits and family h t y of d b t fo th f tu d lo pm nt of manif t di b t

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REFERENCES

- 1 ALPHEUS J I Eva tab 11 5 appl Larned Inf Ings Lec hain 971
- 2 ALPHEUS J I Tr ramp ion f agar Joods I The rdl f agar i ed ra ex m is
Larlagrefraining 7 suppl 9:30 1973
3. ALMEIDA R. FORUM P.M & GONCALVES O Early insulin response intra cases lsa
in steroid-stress diab tes tab lisa 18 2 8 1969
- 4 ALPUCO P P VIE F B & FIAPON H.J The guinea and in arpreta lsa E illi
abnormal oral glucose tol rase Diab tol gra 7 73 1971
- 5 ALPHEUS D I STUTYON F L. VASCHERIL R.J. SIBERTY Intery ta f th rep
intravenous glucose tol rase tes normal and idmal and in mild diab wellitas
J clin. Invest 32 428 1953
- 6 ANDERSON J V KIMBLE R B & HARTY D Effect f high glase and igt ad se di to
glase tol rase f normal ad lsa J lin. Hwr 26 600 1973
- 7 Arterial hypertension ad and ischaemic heart d sease Preventive aspects 16 With Dr tasha
Rep 3 2a 231 1962
- 8 BACHARD J.B. BLOOMER E L. FOWLE J D The significance f basal insulin l vels in
the valuation f the insulin response to glucose i diabetic and nondiabetic subjects
J lin Invest 6 1549 1967
- 9 BACHARD J.B. BLOOMER E L. & FOWLE Jr D Influence f obe ad th relati lisa be wren
urilia and triglycerid vels in adequate hyperting on. idemia. labe 23 664 1971
- 10 BAKER, L. KATE, R.A. POOT Tr earl partial remission of j vasil diab to wellitas
The re f insulin and growth hormone J Pedis 71 825 1967
- 11 BASTIEN J. CORNARD FRANCHES J R.H. HILLES R. & HILBERT, Expt ralia d
late pichabétique Bull Acad roy 68 Belg 31 85 1963.
- 12 BERNHARTI A. STUPPIN G. OCHSKE G.C. FOWLER, J.L. Exaggerated insulin response to
glucose in simpl ab Diabetes 6 666 1967
- 13 BERNHARTI Lachman heart disease in women. A study based on random red population
simpl wren and wren with myocardial isaration in Goteberg Sweden lsa and and
supp 549 1973
- 14 BERNHARTI BLOOMER, O. HILLES L. HILBERT LARSEN E K.P.J.-HILBERT, E
FRO O THILLY E. THILLY WESTERHJ E The Study of Women in G. Umeburg 1968
1969 population loby General design, purpose and sampling results: lsa and cand
93:31 1973
- 15 BERNHARTI E THILLY E BLOOMER GUSTAFSSON I ran shol steral and wren trigl
carides in mild e-aged wren The ad f wren in Goteberg 1968- 1969 Scand J clin lab
Invest 1967 f publication.
- 16 BIR L. & HANSON Body composition and nutrition of school children with cerebral
paly in lsa in rebral paly of ad wren, nutrition and physical working
simpl j school age lsa pedia. cand supp 204 41 1970
- 17 BLANCHARD G. HILLES E P rial and peripheral vein im-arens to arlia same bralies
to are and after wren infarction labe 19:202 1970
- 18 BLANCHARD E. HILLES rial vein insulin concentrations in diabetic subjects
Diab tes 20 284 1971
- 19 BOWEN C.WILSON ALFAR R.E. SIBERT R.J. KEE E. Oral glucose to rase
and related arter normal opalation may Blood sugar plasam insulin glycerid
and the arterial osurens to and the effects of age and sex. Bri med J 335 1969
- 20 BOWEN R.A. lsa thal theory and method loby in science and engineering 2 of John
11 ad lsa Rev ark London and yds 1965
- 21 BOWEN R.A. JAY L. HILLES E FOWLE BIRMAN R.L. Improved g wren
to crase with high carbohydrate f eding in mild diabetes New Engl. J 4 284 321 1971
- 22 BOWEN R.A. KIMBLE T The sul response to glucose comparison to ven oral
and intravenous rase tests J Endour 39 3. 1967
- 23 BOWEN R The lary hi lary ad tes in research. J Amer di 2a 23 041 1967
- 24 BOWEN R.A. L. C.WILSON FENL R.E. ofusion of ra pascanti li rse in
vtr alstra modification of theophylline-induced iphal insulin release J 1 I ves
1970

- 49 DAYKE, H OMOTO W OWEN P.A. & LIEBERMAN H Far South & West Pacific
Diabetes Mellitus: A review and the future of research on the relation of diabetes mellitus to
the 95 983 1970
- 50 BUCHHEIT T LARSEN H & HADSEN, H & HOUJSEN P Insulin response to glucose
tolerance test and isoprenaline in maturity-onset diabetes mellitus Dan Med Bull
19 222 1972
- 51 Diabetes mellitus Report of WHO Expert Committee on Diabetes Mellitus 1965
52 Diabetes Source Book U.S. Department of Health, Education and Welfare Publ. (Natl. Serv.
Publ. No. 168) = H 1968
53. DRAPER J.P. SMITH H Appl. of regression analysis John Wiley & Sons Inc. New York
London & Sydney 1966
- 54 DREYER E J MICHAEL A.S. & GOLD E.H. Biochemical basis of the obesity-hyperuricemia
relationship of liver and kidney metabolism of hypoxanthine. Ann N.Y. Acad. Sci.
25 746 1972
- 55 DUCRAY L.J.P. The insulin-glucose tolerance test Quart J Med Physiol 1 1 5 1956
- 56 DICK D.L. & FODOR J.A. & high-dose insulin as glucose tolerance test J Clin Endoc
26 1032 1966
- 57 EDELMAN H FRANKSON B NICOLAIDES P & KESTEL A F Heding af vurdering af
p. analysing af de forskellige typer af diabetes mellitus af de danske Universitetslaboratorier tryk
gave tryk Del 1971
- 58 ELLIOTT J.P. RASO B.E. Sugar and acid in the oral portion of fruits J Amer Diet
As 90 130 1967
- 59 EMMETT, E.H. & BARKER D Immunological assay of insulin in plasma of children Diabetes
3 77 1964
- 60 EMMETT D Hjortshøj 1. Boleberg 1968-1970 Forbudsloven og diabetes. Karakteristika i
f. de forskellige typer af diabetes og den mulige rolle for den enkelte patient. 97
- 61 Energy and protein requirements Report of J Int FAO/WHO Ad Hoc Expert Comm. on the
Energy Needs Pop. Sci. No. 323 1973
- 62 EMMETT P.S. & RAMES H.H. Diabetes 1 1966 in 1st survey Diabetes 14 212 1965
- 63 EMMETT P.S. Hypertension & risk factor in coronary heart disease Circula ex 36 604 1967
- 64 FALLEN S.B. What is diabetes? Definition, diagnosis and course Med Clin N Am
53 793 1971
- 65 FIELD, P. MILLER E & CANNILL J O.P. Plasma glucose and insulin secretion
in obese J Am Med Assoc 189 1969
- 66 FIELD J.S. Insulin resistance in the obese Handbook of Physiology sec 14 7 Endoc 1
in 1972 505, Westinghouse 1972
- 67 GERTNER S The insulin response in diabetes mellitus Studies of insulin secretion in the
1 1 6 1970
- 68 GERTNER S Pathologic changes of the islets in man Diabetes 1 1007 1972
- 69 GERTNER S. POTTI Determinants of basal and stimulated insulin secretion in man In Handbook of
Physiol. sec 14 7 Endoc 1972
- 70 GERTNER S. LARSEN, H. PAVLATO P.C.H. & OPIKAR P.H. Serum-insulin response to the
1st oral glucose tolerance test Diabetes 1 1970 965
- 71 GERTNER S. LARSEN H. GERTNER S. & GERTNER L. Two-compartmental model of insulin
secretion in the obese J Clin Endoc 32 1970
- 72 GERTNER S. LARSEN H. GERTNER S. Diabetes Sweden A clinical-statistical study of
diabetes and its complications study of many patients in and death certificate data and blood supply
1972 96
- 73 GERTNER S. LARSEN H. Pathogenesis of diabetes mellitus in a normal population Nord Med 2 98 1970
- 74 GERTNER S. LARSEN H. The course of diabetes with insulin-antibody response Diabetes
1972 96
- 75 GERTNER S. LARSEN H. GERTNER S. & GERTNER L. Endocrinological and pathological
study of diabetes mellitus in the elderly Standart re. 1972 1970
- 76 GERTNER S. LARSEN H. GERTNER S. & GERTNER L. The course of diabetes mellitus in the elderly
1972 96

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Supplementum 567

Alcohol Consumption in Relation to Factors associated with Ischemic Heart Disease

A co-twin control study

By Mårten Myrhed

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ALCOHOL CONSUMPTION IN RELATION TO FACTORS ASSOCIATED WITH ISCHEMIC HEART DISEASE

A co-twin control study

By

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"En mängling uppmärksamhet, riktad till sjukdomar hvilka förekomma hos dem, som öfver mått begagna alkoholhaltiga drycker, har lemnat mig en rikströckt erfarenhet, huru mågtiga dessa drycker äro, uti att undergräfvat människans såväl kroppsliga som moraliska hälsa

Magnus Huss, M.D., 1849

Professor of Medicine, Karolinska Institute

Head of the Department of Medicine

Sensuimodestitet

Many years interest in diseases in persons inclined towards excessive consumption of spirituous liquors has provided me with wide experience of the power of drink to enfeeble both the physical and moral fiber of mankind.

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Abbreviations and Terminology

BSA	Body surface area
ECG	Electrocardiography
IHD	Ischemic heart disease
IVGT	Intravenous glucose tolerance test
OGTT	Oral glucose tolerance test
SALP	Serum alkaline phosphatase
SGOT	Serum aspartate aminotransferase (formerly called serum glutamic oxaloacetic transaminase)
SGPT	Serum alanine aminotransferase (formerly called serum glutamic pyruvic transaminase)
SGT	Serum <i>gamma</i> glutamyl transpeptidase
SVEB	Supra ventricular ectopic beat
VEB	Ventricular ectopic beat
U	Unit
Concordance	The occurrence of a trait in both members of a twin pair
Discordance	The occurrence of a trait in only one member of a twin pair
MZ	Monozygotic; monozygosity Twins originating from one fertilized ovum, thus identical in genetic make-up
DZ	Dizygotic; dizygosity Twins originating from two separately fertilized ova, thus genetically related as two ordinary sibs
Pooled zygosity group	Monozygotic and dizygotic twins analysed together
HAG	High alcohol group (for definition see p 14)
LAG	Low alcohol group (for definition see p 14)
M	Mean
SD	Standard deviation

Introduction

Cardiovascular disease has long been the major cause of death in most Western countries accounting for approximately 50% of the deaths in men 50 to 64 years of age. About two-thirds of these deaths are caused by IHD (Blörck 1963 1968, Blörck *et al* 1970 Stamler 1973 Vedin *et al* 1973). So epidemiologic research has concentrated on a search for different factors associated with cardiovascular diseases. Some risk factors (Simborg 1970 Fejfar 1972) are mainly genetic, others mainly environmental, but most are a combination of both. Smoking, which has become a major concern of modern medicine, appears to be influenced by genetic factors, although the habit may be regarded as an environmental trait linked to a genetic constitution (Fisher 1958 Friberg *et al* 1959 Cederlöf 1966, Liljefors 1970).

The present study concerns another of man's habits, a habit which is far older than smoking, the imbibing of alcoholic beverages. This habit has attracted the attention of both medical and public opinion over the centuries and has often been regarded as a threat to man's ability to live well. To date, many investigations have been made on alcohol consumption in relation to health. However most of these have concentrated on problems of etiology prevalence, care and social and psychiatric aspects of alcoholism, rather than morbidity and mortality among alcoholics, a subject not systematically investigated until recently. Even then, somatic investigations were generally carried out on vagrant alcoholics, i.e. subjects suffering from prolonged intoxication and pronounced social and physical deterioration. This implies that excessive drinkers in apparently adequate social

circumstances and maintaining a normal life have been ignored. Furthermore, such research has been mainly into causes of death with the most obvious associations with alcohol ingestion, such as cirrhosis, cancer of the upper respiratory and digestive tracts, accidents and suicide. The increased mortality due to degenerative cardiovascular disease demonstrated in association with alcoholics has only recently been given the attention it deserves.



There are early references in the literature to the cardiovascular effects of alcohol. In 1849 Magnus Huss noted that the blood vessels of an alcoholic are subject, "to the so-called atheromatous process, whereby there is peculiar deposition beneath their innermost membrane, giving rise to the development of a variety of other changes, such as ulceration, aneurysms, rupture and angioliths. At the end of the 19th Century alcohol was regarded as one of the causes of atherosclerosis. Cabot presented data in 1904 indicating that atherosclerosis was actually rare in alcoholics dying before the age of 50. This conception soon predominated and was furthermore supported by a number of studies conducted in the middle of this century. On the basis of material comprising 782 cases of portal cirrhosis, Hall *et al* (1953) was able to report that atherosclerosis in the aorta of males was slightly less than and in the coronary arteries, only half as frequent as in a control group. Grant *et al* (1959) found that only 3.3% of the subjects with portal cirrhosis verified at autopsy had died of acute coronary occlusion with myocardial infarction as compared to 11.4% in the general autopsy population. Howell and Mason (1960) reported that recent or old myocardial

Risk factors defined here as characteristics, traits and abnormalities which are statistically associated with an increased incidence of subsequent IHD.

Infarction was less than one fourth as common in autopsies of subjects with portal cirrhosis as in non-cirrhotic patients. Similarly Ruebner *et al* (1961) found myocardial infarction in 3 % of 399 subjects with portal cirrhosis at necropsy as opposed to 11 % in the same number of controls matched for age, sex and race.

Medical opinion on portal cirrhosis and IHD current in the early sixties was summarized by Stare (1961) who concluded "Whatever the mechanism may be, portal cirrhosis is accompanied by a sparing of the vascular intima, particularly of the coronary circulation. However this opinion was opposed at about the same time. In reporting material from Garland, Bärck (1963) suggested that minute dysfunction of the liver may perhaps promote coronary heart disease: major damage, as in old alcoholics, may protect against it. In a study of 133 cases of sudden IHD death, Bainton & Peterson (1963) found that 36 subjects (27 %) were frank alcoholics or habitually consumed at least 30 oz. (85 cl) of whiskey or its equivalent per week. These results failed to disclose any prophylactic value of alcohol against IHD. An electrocardiographic study by Nielsen & Kensing (1965) of 100 subjects with definite signs of portal or postnecrotic cirrhosis was unable to confirm an increased frequency of signs of IHD compared with a matched control group. Hirst *et al* (1965) objected to the extension of conclusions based on findings in cirrhotic patients to alcoholics in general. They compared the frequency of atherosclerosis and myocardial infarction in cases of chronic alcoholism with and without portal cirrhosis and found that alcohol consumption gives no protection against atherosclerosis but that cirrhosis may do so.

The number of investigations pointing to a link between alcohol consumption and IHD has increased since 1965. Davies (1965) reported that death from atherosclerotic and degenerative heart disease is 2.63 more frequent in alcoholics than in controls. The mortality ratio for Sundby's

(1967) comprehensive study showed that death from IHD was 1.73 more frequent in 1722 treated alcoholics than in the general Norwegian population. The non-vagrant group showed consistently higher mortality rates than the "vagrant" group throughout all the years of the observation. Sundby concluded that alcohol does not "have any beneficial effect upon the degenerative vascular progress. The evidence rather points to an opposite effect, whatever the explanation. Significantly increased IHD mortality ratios in alcoholics have also been reported by Schmidt & de Lint (1972) and Pell & D'Alonzo (1973). "Known, suspected and recovered alcoholics in the latter investigation were carefully matched with controls with respect to age, sex, income group and geographical location. This sample was repeatedly followed for five years, and it could also be shown that hypertension was found 2.3 times more often in the alcoholics than in the controls.

Subjects surviving myocardial infarction also appeared to consume more alcohol than average. In a subject group comprising 72 males surviving myocardial infarction Villiger & Hayden (1966) found that 74 % of the subjects satisfied the criteria for major or minor alcohol abuse as opposed to 28 % of the control group. In a Swedish study of men born in 1913 Tibblin (1972) found that the incidence of IHD over a 9 year period in the group with alcohol problems—defined as registration at the local Temperance Board*—was 3 times greater than in the complementary group. The mortality rate for registered smokers was more than 6 times higher than for non-registered non smokers. Analysing Tibblin's material (1967/1972) by means of a multiple logistic model, Wilhelmsen *et al* (1973) found that registration at the Temperance Board, which represents a rather rough indication of drinking habits, was an independent risk factor for acute nonfatal myocardial infarction and acute fatal IHD. Friberg *et al* (1973) reported that 10 % of non smokers were registered in a nationwide alcohol registry as opposed to 30 % of those smoking more than 10 cigarettes a day. The data suggest that IHD mortality among registered twins,

*Sw. Nykterhetsnämnden
Sw. Kreninalshälseregistret

irrespective of their smoking habits, was higher than among the non registered twins.

☆

The importance of heredity in the pathogenesis of IHD is much debated, but family and twin studies, have shown it to play an important role (Epstein 1964, 1965; Lundman 1973). Thomas (1938-1939) studying the parents and grand parents of 724 medical students, found that cardiovascular disease was 2.7 times more common when both parents were affected as compared with offsprings of non-affected parents. Russek & Zohman (1958) obtained a history of definite cardiovascular disease in one or both parents of 67% of coronary patients as compared to 40% of the controls. Among the parents of males who had suffered a proven myocardial infarction, Rose (1964) found the IHD mortality rate to be 3 times higher than in matched controls. In a prospective study of a large number of subjects, Hammond *et al* (1971) found that IHD mortality rates were considerably higher among subjects with short-lived parents than among subjects with long lived parents.

However it must be remembered that familial aggregation of IHD is no proof of any genetic mechanism. Twin studies are better suited the task of establishing the existence of genetic factors in IHD. Monozygotic (MZ) twins have genetic factors completely in common, while same-sexed dizygotic (DZ) twins have them to approximately 50%. Furthermore, twins tend to share environmental factors to a greater degree than other groups (Cederlöf 1966; Lundman 1973). Verchuer (1958) found a concordance rate of 19% for coronary sclerosis among 21 pair of MZ twins as compared to 8.5% in 47 pair of DZ twins. Harvald & Hauge (1965) analysed data from 6,893 pair of twins in Denmark. Concordance with respect to coronary occlusion was found 70% of the MZ twins as compared to 15.5% of the same-sexed DZ. However, the difference was not significant. In a study of 92 MZ pair despite discordant smoking habits in the twin pairs,

Lundman (1966) was able to show a coincidence rate of 18.5% with respect to clinically evaluated or exercise ECG-diagnosed IHD. The anticipated coincidence was 9.6%. A significantly higher concordance rate for angina pectoris was found among MZ than among DZ twins (Cederlöf *et al* 1967) in the Swedish Twin Registry (Cederlöf 1966). Liljefors (1970) also found a higher concordance rate for MZ than for DZ twins when ST-segment depression during exercise was included as a criterion of IHD.

☆

Alcoholism has long since been observed to run in families. Both Amarik (1951) and Wnour *et al* (1970) studying the alcoholic incidence in first degree family members of alcoholics, found alcoholism rates exceeding those anticipated for the general population. Schuchat *et al* (1971) performed an investigation utilizing half-siblings of alcoholic parents. The results indicated that the presence of a biological, alcoholic parent predicted alcoholism in the half-sibling sample, while living with an alcoholic parent figure did not.

Twin studies dealing with alcoholism and alcohol consumption have been performed by Kaij (1960) who published a report on 174 male twin pairs in Sweden. At least one twin in each pair was an alcoholic registered at the Temperance Board. The concordance rate for alcohol consumption in the whole twin sample for MZ versus DZ twins was reported at 54 and 28% respectively. As the degree of alcohol abuse increased, the concordance rate was found to increase among the MZ but not among DZ twins. Partanen *et al* (1966) used a cohort of Finnish twins for a study of alcohol consumption and alcohol habits in 902 male twin pair. They concluded that there is a heritable influence determining the frequency and the amount of drinking in the sample and a "lack of control" for the younger group, but there were no differences between MZ and DZ twins with regard to social complications. From answers to a questionnaire mailed to 1,500 same-sexed twins Johansson & Nilsson (1968) found that both a

large consumption and abstinence were influenced to some extent by genetic factors. Thus, the three studies reported are consistent with a genetic influence on drinking patterns.

☆

Great interest has been devoted to the relationship between *excessive* alcohol consumption and specific somatic responses. Recent studies, using an epidemiological approach involving large numbers, noted that subjects with often extreme alcohol consumption were more prone to develop IHD. The purpose of the present study with its clinical approach was to examine the extent to which moderate, longstanding alcohol consumption is accompanied by somatic deterioration and if it gives rise to factors associated with IHD. Overt IHD has a relatively low prevalence in middle aged Swedish men (e.g. Tibblin 1967, Björck 1968, Stamler 1973). Consequently its investigation in such a population would require a very large number such as the Twin Registry can not yet provide. Hence, the prevalence of precursors of IHD has been investigated. As genetic factors have an influence on the development of IHD it has been considered important to minimize the in-

fluence of the hereditary background as much as possible. Therefore a subject group comprising twin pairs was selected i.e. twin pairs differing with respect to alcohol consumption. Few alcohol-discordant monozygotic pairs have been traced, since monozygotic twins have shown high concordance in alcohol consumption. However since about 50 % of genetic factors are shared in common, same sexed dizygotic pairs are also of considerable value. In addition to this advantage, the twin approach also means that the influence of early environmental factors is reduced. Thus, a twin comparison must be considered superior even to comparisons based on matched pairs. The hypotheses set up for the present study were the following:

1. Moderate longstanding alcohol consumption is accompanied by a gain in weight, an increase in subcutaneous fat thickness, high tobacco consumption, elevated blood pressure and elevated values for serum lipids, blood glucose (with a pathological intravenous glucose tolerance test) and serum uric acid.

2. There is a connection between longstanding, moderate alcohol consumption and IHD diagnosed according to the case history and electrocardiographic examination.

Twin methods and selection of subject group

The special properties of twin populations in medical research provide exceptional opportunities for control of hereditary and environmental variables. Monozygotic (MZ) and dizygotic (DZ) twins would probably even share a common environment to a higher degree than other subjects. Control opportunities of this kind can not be achieved with groups selected at random.

THE CLASSICAL TWIN METHOD

The classical twin method is based on the assumption that members of any MZ twin-pair share their common environment to the same extent as members of any DZ twin-pair. If this is the case any resemblance between MZ twins which is greater than the resemblance between DZ twins must be ascribable to genetic factors. Furthermore any differences between the members of a MZ pair are considered to be the result of dissimilarities in their environment. The rationale of this approach has been discussed by e.g. Ostlyngen (1949) Gedda (1961) Harvald & Hauge (1965) and Cederlöf (1966).

The classical twin method has not escaped criticism. It has never been proved that environmental intra-pair differences are the same for MZ and DZ twins.

THE TWIN CONTROL METHOD

In 1942 Gesell introduced an experimental method which he called "the twin control method". The method involved the exposure of one of the MZ twins to certain treatment, the unexposed co-twin serving as a control. This was a clearest experimental method using "matched pairs" and manipulating stimulus factors. Since MZ twins have the same genetic origin, there were no constitutional differences between subjects and con-

trols. Glass (1954) has used the method on a small scale in pharmacological experiments. Dencker (1958) studied the sequelae of head injuries affecting one MZ twin and used the un-affected co-twin as a control. The co-twin control method has been used relatively seldom, despite its many advantages.

A different situation was found in studies of smokers and nonsmokers conducted by Cederlöf *et al* (1966) and Lundman (1966) who compared the frequency of certain chronic disorders in exposed and non-exposed partners. Smoking can not be regarded as a randomly induced habit but results from "self-selection". Hajj (1960) also used the co-twin control method to study the occurrence and structure of chronic alcoholic deterioration by comparing MZ and DZ twins with discordant drinking habits. Liljefors (1970) studied the frequency of factors associated with IHD in IHD-discordant twin pairs. Thus, application of the co-twin control method in this manner is only partly analogous to an experimental model.

To sum up twin studies possess two general advantages: firstly the classical co-twin method provides the investigator with information as to whether a disease has a genetic influence, and secondly if this is the case makes it possible to keep hereditary factors constant when the effect of specific environmental exposure is evaluated.

THE SWEDISH TWIN REGISTRY

The present group of subjects was assembled using the Swedish Twin Registry compiled in 1961—1962 at the Department of Environmental Hygiene, Karolinska Institute and the National Institute of Public Health. The registry comprises about 10,000 sets of same-sexed twins (about 4,500 male and 5,500 female pairs) and covers about 95% of all Swedish same-sexed twins born

TABLE 1 Distribution of examined 1 in pairs by age and zygosity

	AGE				M(SD)	Total
	45-50 yrs	51-55 yrs	56-60 yrs	61-65 yrs		
MZ	2	4	4	4	36.9(3.2)	14
DZ	15	19	17	5	34.6(4.6)	56
MZ+DZ	17	23	21	9	34.6(4.8)	70

in the country from 1886-1925 and still alive at the time of complication. About 35% of the twin pairs were judged to be MZ. The registry has been presented in detail by Cederlöf (1966) and Cederlöf *et al* (1970). Data were mainly collected using three different questionnaires; mortality is registered continuously (Friberg *et al* 1970, Friberg *et al* 1973). Two clinical studies of sub-samples have also been made (Lundman 1966, Liljefors 1970).

Information was obtained from the questionnaires, mailed to the entire twin population, on zygosity, smoking, angina pectoris, respiratory symptoms and a number of socio-economic variables. Information on alcohol consumption was also included. The present group of subjects was selected on the basis of this information. The questions pertaining to alcohol consumption originated from a questionnaire originally distributed in 1967. The mailed questionnaire, returned by the twins in 1967, consisted of questions involving the frequency and amounts of certain types of alcoholic beverages consumed. Thus, consumption was based on a frequency-quantity index, expressed in grams of absolute alcohol (Appendix, page 92).

CRITERIA FOR SELECTION AND THE SUBJECT GROUP

The material was classified according to the following drinking discordances, based on the answers of the 1967 questionnaire: >10 000 <2,000 5 000-10 000 0-400 and 2,000-5,000 grams of absolute alcohol/year: no consumption whatever. About 70% of the target

male population answered the 1967 questionnaire. In total 86 male twin pairs, approximately 25% of whom MZ and 75% DZ twins, of slightly more than 1 700 pairs, fulfilled the first criterion. Another 83 twin pairs fulfilled the other two criteria. All of the 86 highly discordant twin pairs were invited to take part in a clinical examination at the Serafiner Hospital. Another 6 twin pairs living in the Stockholm area who fulfilled the second criterion above and showed a high level of alcohol discordance, were also included in the examination. According to these criteria the members of each pair were assigned to a high alcohol group (HAG) or low alcohol group (LAG). A total of 92 pairs, from 45-65 years, were invited to participate in the study and 70 (76%) complete pairs were examined. Fourteen of these pairs were MZ and 56 DZ twins. The distribution of the series with respect to age and zygosity is shown in Table 1.

Comments

Even though the Swedish Twin Registry provided a large material for selection, only 86 potentially alcohol-discordant twin pairs could be found. That the number is small is mainly due to a high rate of concordance among twins in the answers given concerning alcohol. Alcohol-discordant pairs were especially uncommon among MZ twins. These findings were consistent with the genetic influence on drinking patterns demonstrated in earlier twin studies (Kaj 1960, Partanen *et al* 1966, Jonsson & Nilsson 1968). Another factor which may have affected the selection is the presumed rather high drop-out rate for questions pertaining to alcohol. It is reasonable to assume

that some highly alcohol-discordant pairs were lost on account of this reason.

Only male twin pairs were selected for the study because the number of females with alcohol discordant drinking habits in the Twin Registry was regarded as small, and because more serious forms of IHD such as myocardial infarction and sudden IHD death, are less frequent among women than men (e.g. Epstein 1963 Kannel *et al* 1967b, Sumborg 1970 Kuller & Tonascia 1971 Stamler 1973 Bengtsson 1973).

Since examined twins in ideal circumstances should have the same background according to the area of residence, this background was analysed in the subject group differences were found in 6 pairs. Four of the high consumers resided in urban areas while the low consumers resided in rural areas. The opposite was found in 2 pairs. Thus, no dissimilarities with respect to area of residence could be shown between the members of the twin pairs. This eliminates the environmental influence caused by area of residence said to affect the cardiovascular system (Björck 1959 1963 Epstein 1963 Sumborg 1970 Stamler 1973).

TIME AND PLACE OF INVESTIGATION

The investigation was begun in February 1972 and completed in March 1973. Most members of twin pair were examined in different weeks but, principle, on the same day of the week. All the twins were examined at the Serafimer Hospital, Stockholm.

Each twin arrived at the hospital at 7.45 a.m. and had completed the medical screening by 12 p.m. After lunch, the twins were interviewed by a psychologist and psychiatrist. Interviews took a total of 1–2 h for each subject. Thus, the twins were able to leave hospital between 2 and 3 p.m. Only one twin was examined each day.

Different population studies have shown that subjects with a particular disease are more inclined to submit to medical examinations than others (Cobb *et al* 1957). To avoid this bias, the twins were not informed that this study was concerned with IHD or about the specific effects of alcohol on the cardiovascular system.

NON-RESPONSE

Twenty-two pairs (24%) were not available for study (Fig. 1).

One or both of the twins in 8 pairs had died in 1967–1972. All of the deceased had been subjected to autopsy examination. The causes of death, according to the pathologist's report, are presented in Table 2.

Five subjects died both in the high alcohol group (HAG) and in the low alcohol group (LAG). Four of the subjects in the HAG and the same number in the LAG group died before their co-twins. Diagnoses associated with IHD were found in 3 of the twins in the high alcohol group and in one of the twins in the low alcohol group.

Five pairs did not participate in the investigation because of severe illness. The diagnoses reported were cerebral infarction with hemiplegia, polymyositis, grave epilepsy and debility in two cases. Low alcohol consumption was present in 4 out of 5 pairs among these sick patients.

TABLE 2. Causes of death leading to non-response by alcohol-discordant twins

High Alcohol Group (HAG)	Low Alcohol Group (LAG)
1 Fibrosis myocardii + Atherosclerosis coronariæ	Uremia + Hypertonia + Sepsis (myocardium and coronary arteries NAD)
2 Tumor pulm. c. metastases multiple	Aln
3 Infarctus cordis + Cardiosclerosis	Aln
4 Aln	Insuff. cordis + Hyperthyreosis
5 Strangulatio + Depressio mentis	Aln
6 Aln	Myelomatosis
7 Aln	Bronchopneumonia + Fractura crani
8 Insuff. cordis acutus + Morbus cordis arterioscleroticus	Cancer endriculi c. metastases

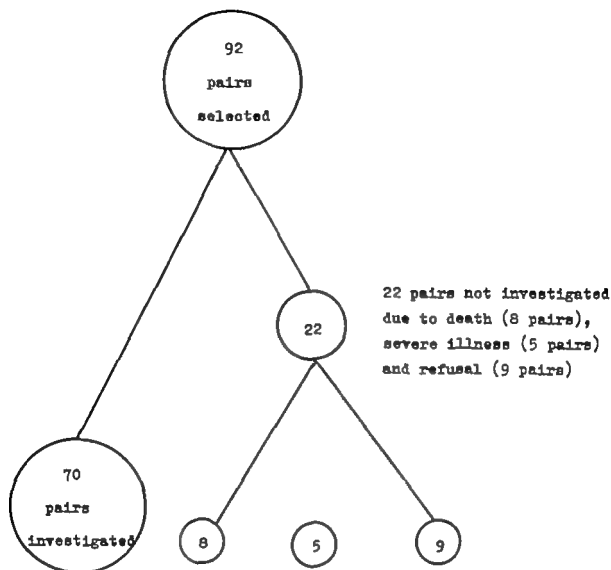


Fig 1 The Subject Group

Only 9 of the 92 (9.8 %) twin pairs refused to take part. In most cases this was because their work presented an obstacle, but unwillingness, fear or the like also played a part.

Comment

The number of non participants must be regarded as low especially when considering that many of the subject had to travel more than 300 miles to reach the examiner. The non respondents

were fairly evenly distributed with respect to age and zygosity as compared to the subject group. It is not possible to be certain how non respondents biased the final results. All of the Irving non-participants were contacted by telephone. In many cases it was obvious that these pairs included a member who was unable to make the journey to the investigator because of alcohol consumption. Therefore, it may well be that the possibility of disclosing differences between the various exposure groups has been reduced.

ZYGOSITY DIAGNOSIS

Zygosity in the Twin Registry has been estimated on the basis of replies to questions relating to resemblance, viz. alike as two peas in pod as children. If both twins agreed that they had been as alike as two peas in pod, the pair was considered to be MZ. If on the other hand, they only admitted to a family resemblance the pair was regarded as DZ. The validity of this method has been examined by Cederlöf *et al* (1961). Zygosity was verified in 95% of the MZ and 90% of the DZ twins by means of serological methods; five different grouping systems were used.

Serological determinations were performed on all twins in the present study. It was carried out at the National Laboratory of Forensic Medicine and comprised the blood group systems ABO, Rh, MN, P, K, Le, Fy, Jk and the serum groups Hp, Gc, Ag, Gm, Inv, PGM, EAP, BA, AL, ADA, and PGDA. When there were differences in zygosity grading between the Twin Registry and the serologic determination, the laboratory investigation was considered to be the most valid. All of the 34 initially DZ pairs had the same zygosity according to serological determinations, and 14 of the 16 initially MZ pairs showed the same serological zygosity. Two twin pairs had been classified as MZ in the Twin Registry but as repeated

serological tests displayed different serum groups, they were classified as DZ in the present study.

Comments

The smaller the subject group, the more important the determination of zygosity. Correct identification of zygosity can only be obtained in pairs in whom a common chorion membrane is found. Serological determination in the present study must be regarded as the most valid method for zygosity determination. The number of blood group systems and serum groups used in the present study group was large, increasing the reliability of adequate zygosity determination. The resemblance between the results obtained by the Department of Hygiene and the National Laboratory of Forensic Medicine strengthened the zygosity diagnosis.

The number of twin pairs with respect to zygosity thus showed 14 MZ and 36 DZ pairs. It is clearly not possible to evaluate the influence of genetic factors with this small material. Nevertheless, the material classified by zygosity groups will be described. The results will be presented in terms of the pooled zygosity group (MZ+DZ). However, it was considered of some interest to see whether or not the small number of subjects in the MZ group would follow the same tendency as found in the DZ group.

General methods and procedure

Case history

In order to avoid investigator bias, each subject's case history was taken after the physical examination and without information of the results of laboratory tests. Nor did the investigator know the individual answers to the mailed questionnaire sent out by the National Institute of Public Health 1967. He was thus completely ignorant of the drinking habits of the examined subjects. The interview consisted of a sociological and a medical part. About 250 different items were discussed. Thus, the attention of the subjects was not focused on questions concerning alcohol.

Sociological interview

The sociological interview was conducted by the author. Standardized questions pertaining to family conditions, education, occupation, work, smoking, diet and physical exercise were included. The amount of alcohol consumed and the pattern of "alcohol behavior" were thoroughly assessed. The questions were more detailed than in the 1967 questionnaire (Appendix, p. 92) to obtain an accurate measurement of present alcohol consumption. The methods used in the evaluation of the alcohol questionnaire are presented in detail in chapter III. Since a separate report on the subject group's sociological, psychological and psychiatric variables is to be published by the Department of Psychiatry, St Goran's Hospital and the Department of Medicine, Serafimer Hospital, Stockholm, no information on these items will be provided in this account. It can be mentioned that no disparities were found between high and low alcohol consumers as to e.g. income and how many of them were gainfully employed. No less than 90% of the investigated subjects in both groups were working.

Medical interview

Before his arrival in hospital the subject had already received a questionnaire about his previous and present state of health. This consisted of a general and a cardiovascular section. The answers were analyzed by the author in an interview to ensure that all questions had been properly answered.

Questions concerning *respiratory symptoms* were in part the same as those drawn up by the British Medical Research Council's Committee on the Aetiology of Chronic Bronchitis (1960).

Cough was diagnosed when morning cough was present regularly.

Phlegm was diagnosed when morning phlegm was present regularly.

Asthma was diagnosed when it had been diagnosed by a physician or when typical evidence of asthma was found in the medical history.

Dyspnea was classified in 5 grades, but the results for grades 1—2 and 3—5 are presented together. Grade 2 denoted breathlessness when hurrying on level ground or waking up a slight hill and grade 3 shortness of breath when walking with other people of same age on level ground (Rose & Blackburn 1968).

The *cardiovascular questionnaire* was in part identical with the questionnaire designed and tested for several years at the London School of Hygiene and Tropical Medicine (Rose 1962).

Myocardial infarction was diagnosed after Hofvendahl (1971).

1. Central chest pain for ≥ 30 min combined with at least one of the following criteria.

a) The appearance of a pathological Q wave and/or the appearance or disappearance of a localized elevation of ST segment, followed by inversion of T waves in at least two ECG leads.

b) Laboratory indications of a myocardial dam-

age: two SGOT values of 40 U or more achieving peak value about 24 h after the onset of symptoms, in combination with lower SGPT values, reaching a peak after about 36 h, and/or two HBD (alpha-hydroxybutyrate dehydrogenase) values exceeding 75 U of corresponding LDH (lactic dehydrogenase) values exceeding 400 U with a peak about 60 h after the onset of symptoms or combination of one SGOT SGPT combination and one HBD-LDH combination, elevated as stated above.

2. Diagnosis at autopsy

Suspected infarction existed if the subject had suffered central chest pain lasting for at least 30 min unaccompanied by the above signs of myocardial infarction (Rose 1962)

Angina pectoris was defined as a pain in the sternal region, with or without radiation into the arms or towards the jaw or pain to the left or anterior chest radiating into the left arm, accompanying physical exertion and disappearing or subsiding within 10 min after resting or taking nitroglycerine medication (Rose 1962)

Chest discomfort existed when some but not all of the aforementioned criteria for angina pectoris were fulfilled.

Palpitations bradycardia and irregular heart rate were considered to exist when reported by the subject.

Intermittent claudication was diagnosed according to Rose (1962)

Suspected intermittent claudication was diagnosed when some but not all of the stated criteria were fulfilled and when intermittent claudication was strongly suspected

Comments

The use of identical questions concerning different symptoms and other items will increase the validity of intra pair comparison, as compared to an unstructured interview (Rose & Blackburn 1966). The diagnoses of angina pectoris and intermittent claudication were based on criteria recommended by Rose (1962) and the WHO Expert Committee on Arterial Hypertension and Ischaemic Heart Disease (Burgess *et al* 1963). In a validity

test, Rose (1961) found that uniform questionnaires greatly reduced intra- and inter-observer variability in the diagnosis of angina pectoris.

Physical examination

The following variables were examined in addition to an examination of general appearance: lymph nodes, the mouth and throat, heart, peripheral arterial pulses, lungs, abdomen, thyroid gland and tendon reflexes.

Anthropometric variables

Weight was recorded to the nearest kilogram and included light underclothes.

Overweight was determined according to a modification of a weight/height index described by e.g. Frisk *et al.* (1959)

weight (kg)

0.9 (height, cm-100)

Skinfold thickness was determined by measurements in the subscapular and triceps areas on the right side of the body. A Harpenden skinfold caliper (Keys 1966) was used. The pressure applied by the caliper was 10 g/mm² and the area of the contact surface was 20 mm². The skin and subcutaneous tissue were gripped with finger and thumb 1 cm above the point of measurement and squeezed firmly. The skin fold was measured to the nearest 0.1 mm.

Blood pressure measurements

The blood pressure of all subjects was measured with the same mercury manometer between 9-10 a.m. The procedure was performed in the same room, and noise and chilling were avoided. The pressure was measured in the right upper arm with the subject in a supine position. An adhesive cloth sleeve measuring 33×13 cm was used. Korotkoff's auscultatory method was used, the systolic pressure being read at the appearance of the sound and the diastolic at disappearance. At least two measurements were performed. If the values were not identical the procedure was repeated, the lowest value then being recorded. The reading was made to the nearest 5 mm Hg.

Blood pressure was recorded at the start of the

examination (casual blood pressure) and after 15 min of rest in complete quiet (basal blood pressure)

Comments

Sources of error in sphygmomanometry can be roughly divided into three categories firstly the design of the cuff and variations obtained with respect to arm circumference and cuff width, secondly the fact that different examiners taking the blood pressure of the same subject may obtain different readings thirdly possible differences in the interpretation of Korotkoff's sounds (Rose & Blackburn 1968 King 1969)

The results of three different studies on cuff dimensions (Karvonen *et al* 1964 Simpson *et al* 1965 King 1969) were in close agreement, indicating that long-bladdered cuffs produced the smallest range of random errors irrespective of arm-circumference. All these studies recommended a cuff containing a bladder at least 40 cm long. The most important factor in indirect blood pressure measurement is intra and inter-observer variability. The latter was eliminated in the present study since all the determinations were made by the same person (the author)

Diastolic pressure in the subject group was measured when Korotkoff's sounds disappeared (Phase 5) Karvonen *et al* (1964) and King (1969) found that this measurement agreed the closest with the values obtained by intra arterial means.

Some somatic definitions

Dupuytren's contracture was defined as a contraction of the palmar fascia.

Erythema of the palms was defined as an exaggeration of the ordinary speckled mottling of the palms.

Gynecomastia was considered present when a fleshy firm disc of tissue with well-delimited borders was palpable in the substance of the breast.

Hepatomegaly was defined as a liver palpable at least 3 cm below the right arcus in the mid clavicular line.

Hypertension was defined as a history of high

blood pressure treated with anti-hypertensive agents.

Tremor was defined as coarse finger tremble with a larger-than normal amplitude found in writing or when the subject held a piece of paper between two fingers.

Vascular spiders—Paper money" skin was defined as more than three spiders or numerous small vessels present in the vascular territory of the superior vena cava.

Jaundice was defined as true or suspected yellow sclerae

Laboratory tests

Blood and urine tests

Blood samples were taken from the subjects in the morning following an overnight fast. A urine specimen was collected the same morning. All analyses, with the exception of blood alcohol, were performed at the Department of Clinical Chemistry Serafimer Hospital. Serum was obtained by centrifugation. E.S.R., serum creatinine hemoglobin, serum aspartate aminotransferase (SGOT) serum alanine aminotransferase (SGPT) serum alkaline phosphatase (SALP) white blood cell count, platelets, serum lipid electrophoresis, blood alcohol and blood glucose were determined. Twenty ml of serum was stored in a freezer for subsequent concomitant analysis of the members of the twin pairs with respect to serum lipids and uric acid. Twin pairs discordant as to > 1000 grams of absolute alcohol/month were also subsequently and at the same time analysed with regard to serum gammaglutamyl transpeptidase (SGT). Investigations for glucose, protein and sediment were made on urine samples.

SGOT and SGPT were analysed using a Reaction Rate Analyser (LKB 8600) connected to an evaluation unit (Optilab Bo Philip Instrumentation, Stockholm). Reagents for SGOT and SGPT were obtained from Kabi AB Stockholm. Reference values. SGOT and SGPT ≤ 55 U/l

SALP was analysed according to Bergström & Thunblad (1970). Reference value SALP ≤ 35 U/l

SGT was analysed according to Szasz (1969). Reagents were from Roche Diagnostics, Basel. Reference values ≤ 40 U/l.

Blood glucose was determined in capillary blood samples from the finger tip using a glucose oxidase method. Reagents were GLOX NOVUM obtained from Habi AB, Stockholm, 1972. Reference values 55–100 mg/100 ml.

Chol sterol was determined with an Autoanalyzer according to the method of Levine & Zak (1964). Reference values 140–290 mg/100 ml.

Triglycerides were determined with an Autoanalyzer according to the method of Keisler & Lederer (1965). Reference value < 180 mg/100 ml.

Lipoproteins in electrophoresis in agarose gel was carried out in accordance with a method described by Noble (1968). Normal range: Alfa lipoprotein 25–50%, beta lipoprotein 40–60%, pre-beta lipoprotein 5–25%.

Uric acid was determined with an Autoanalyzer according to the method of Hawk *et al* (1964). Reference value in males < 7.5 mg/100 ml.

Blood alcohol was determined, according to Goldberg & Rydberg (1965) at the Department of Alcohol Research, Karolinska Institute, Stockholm.

Glucose tolerance test

The intravenous glucose tolerance test (IVGT) was used, as described by Wahlberg (1966). Participants were asked not to eat nor take any medication after 8.00 p.m. the day preceding the test. All tests were started at 8.00 a.m., and subjects rested for at least 15 minutes before the start of the test.

After taking duplicate capillary blood samples for glucose determination, 25 grams of glucose in a 50 cc aqueous solution were injected intravenously over a period of 2 to 4 min. Zero time was set at the end of the injection. Blood samples were then taken every 10th minute for 60 minutes, the samples at 20, 40 and 60 minutes duplicated. The subjects were kept supine throughout the test. The half life of blood glucose in minutes ($t_{1/2}$) was determined with an electronic desk calculator

(Hewlett Packard) which also calculated the k value according to the equation previously described by Ikkos & Luft (1957), Wahlberg (1966) and Passikivi (1970).

$$k \text{ value} = \frac{0.693 \times 100}{t_{1/2}}$$

In accordance with the two latter authors, the following IVGT classification was used.

Diabetic IVGT k value	≤ 0.90
Borderline	$0.91-1.00$
Normal	≥ 1.11

Comments

The IVGT was chosen instead of the oral glucose tolerance test (OGTT) for the following reasons. The IVGT had been used at Serafimer Hospital since 1960 and found to be reproducible in patients over intervals of days and years (Passikivi 1970). Furthermore, the test is simple to perform and less time-consuming than the OGTT. Also the results are easy to express and convenient for statistical analysis. The use of a desk calculator for estimation of k values probably avoided methodological errors in the original graphical technique.

Chest X-rays

A chest X-ray with the patient standing was taken according to Liljestrand *et al* (1939). The relative heart volume, expressed in ml/m² of body surface area (BSA) was calculated (Jonzell 1939) by an experienced radiologist (Leif Erman, M.D.) who also examined the heart configuration and lung films. The upper limit for heart size in men is 500 ml/m² of BSA, at the X-ray Department of Serafimer Hospital.

Electrocardiographic examination

The clinical examination included ECG records at rest and, in all cases but one, during exercise. The recordings after exercise were made immediately 3 and 10 minutes after the test. The instrument was a direct recording 6-channel electrocardiograph (Mingograf 61 Elema Schöander AB Stockholm). The ECG before and after work was recorded with the subject in supine position, and

the following leads were used I—III, aVR, aVL, aVF and 6 chest leads (CR₄R, CR_{1,2,4,5,7}). The chart speed was 50 mm/s.

The exercise ECG was recorded with the subject exercising on an electrically braked bicycle ergometer (Elema-Schöander AB Stockholm). The initial load was always 50 Watts. The load was increased stepwise at 6-min intervals until the subject was exhausted, developed angina pectoris or showed ECG changes (arrhythmias, ST-segment changes etc.). To avoid interference from muscle action potentials during the test, the reference electrode was placed on the forehead according to Holmgren & Strandell (1961). During exercise the ECG was recorded using a chart speed of 10–25 mm/s. The heart rate was calculated from the ECG.

Interpretation of ECG

The ECG findings were coded according to a modification (Astrand *et al* 1967) of the Minnesota code (Blackburn *et al* 1960).

The ECG was interpreted at the highest comparable heart rate so as to facilitate intra-pair comparison of the ECG response during exercise. This was defined as the highest heart rate recorded by the twin with the lower final heart rate in a pair. All ECG examinations were interpreted by the author over a continuous period of about 2 weeks and without any knowledge of the subjects' identity. Several ECG recordings were coded twice to ensure that the criteria had not changed during the period of interpretation. Torbjörn Lundman, M.D. kindly interpreted several of the ECG tracings to confirm that the interpretation agreed with that of an experienced cardiologist. In accordance with Blomqvist (1965, 1971) and Astrand (1973) segmental ST depressions according to ST code 1–3 (Table 3) were considered indicative of subclinical IHD.

Comment

Inter-observer variations are greater than intra-observer variation in ECG interpretation (Astrand 1965). She found close agreement for ST depressions according to ST code 1–3 and slightly

TABLE 3 *Electrocardiographic codes for ST depressions*

Code number	ST junction and segment measured in leads I, II, VI, VF CR _{1,7}
0	Normal ST segment
1	ST J depression of at least 1.5 mm or at least 1.0 mm and ST segment straight and slowly ascending, horizontal or downward sloping
2	ST J depression of 1.0–1.4 mm and ST segment straight and slowly ascending, horizontal or downward sloping
3	ST J depression of 0.5–0.9 mm and ST segment straight and slowly ascending, horizontal or downward sloping
4	ST J depression less than 0.5 mm but ST segment downward sloping and reaching 0.5 mm or more below P R baseline
5	ST J depression less than 0.5 but ST segment horizontal or downward sloping and reaching less than 0.5 mm below P R baseline
6	Isolated ST J depression of at least 1.5 mm or at least 1.0 mm ST segment upward sloping
7	Isolated ST J depression of at least 0.5–1.4 mm or at least 0.5–0.9 mm ST segment upward sloping

poorer results according to ST code 4, in repeated coding of 200 ECG records.

Statistical methods

Conventional statistical methods have been used for the calculation of the arithmetic mean (M) and standard deviation (SD).

With respect to intra-pair comparison of qualitative variables, such as symptoms, diagnoses and ECG-findings, concordant and discordant pairs were expressed in absolute numbers, as the example given below:

		Low alcohol group	
		Affected	Un-affected
High alcohol group	Affected	a	b
	Un-affected	c	d

The statistical analysis refers to the cells b and c which consist of the numbers of twins discordant with respect both to alcohol consumption and to the dependant variable. Cell b expresses the number of twins in the high alcohol group positive to a trait when a co-twin is not. Cell c on the other hand, expresses the number of twins in the low alcohol group positive to a trait when a co-twin is not. The assessment of statistical significance deals with the relationship between these two figures. The one tailed hypothesis that the twins in the high alcohol group had a greater prevalence of positive findings was tested using McNemar's test (Siegel 1956). The Binomial test was used in cases with less than 10 discordant twin pairs (Siegel 1956).

The mean intra pair differences for quantitative variables in relation to alcohol consumption were examined using Student's paired "t"-test. The following equation was used

$$t = \frac{\bar{d}}{\sqrt{\frac{SD^2}{n}}}$$

in which \bar{d} is the mean of intra-pair difference, n the number of pairs observed and SD_d the standard deviation of the differences between the pairs of observations. Degrees of freedom are $n-1$.

It is not self-evident that quantitative variables are statistically best evaluated by using an intra pair difference method. It might well be that a small number of pathologically altered values may only affect mean group values in a minute degree while increasing variance considerably. In cases in which conventional cut-off points between normal and pathological conditions can be defined, a qualitative assessment may be statistically more efficient. Many quantitative variables were also subjected to qualitative assessment in the present study.

The null hypothesis of no difference was rejected at the significance level of 5% ($p < 0.05^*$), 1% ($p < 0.01^{**}$) and 0.1% ($p < 0.001^{***}$).

Evaluation of alcohol discordance and validity of alcohol classification

The twins in the 1972/73 investigation were carefully interviewed with respect to alcohol consumption and alcohol habits in an effort to analyse the validity of the information obtained from the 1967 questionnaire (Appendix, p. 92). Furthermore, physical findings and some determinations of serum enzymes associated with a high alcohol intake were also investigated.

Alcohol in blood was determined (p. 21) to check the fasting status. Only one subject had detectable alcohol (1.4 / ∞). He belonged to the high alcohol group.

ALCOHOL EVALUATION

One bottle of beer (33 cl) was assumed to contain 12 g of absolute alcohol, one bottle of wine (75 cl) 60 g and one bottle of spirits (75 cl) 250 g (See Appendix, p. 92). Thus a subject reporting the consumption of one beer daily was assumed to have an annual consumption of $365 \times 12 = 4,400$ g of absolute alcohol (Wallgren & Barry III 1970; Hollstedt 1974).

Comments

The 1967 questionnaire was unsatisfactory as insufficient alternatives were provided for the frequency of alcohol consumption. Thus, just one alternative was provided—once a week—for respondents who might drink once, twice or three times a week. When selecting twin pairs, this alternative was assumed to be twice a week. This often produced too high an estimate of a subject's consumption. Furthermore, no distinction was made between the different types of beer now available in Sweden. Weak beer (class I) for example, only contains 6 g of absolute alcohol per bottle

(33 cl). In the following alcohol discordance was consistently based on the last interview i.e. the one carried out in 1972—1973. This means that the information on alcohol consumption in the 1967 questionnaire was only used to select alcohol discordant twin pairs.

REPEATABILITY

A comparison of intra pair differences expressed in grams of absolute alcohol in 1967 and 1972/73 is provided in Table 4. Forty-two of the 70 pairs investigated (60 %) in 1972/73 still displayed a discordance of more than 8 000 g of absolute alcohol/year. This difference pertains to the primary criterion for selection ($> 10\,000$ versus $< 2\,000$ grams/year). In 1972/73 a total of 57 pairs (81 %) displayed a difference in consumption of at least 4 000 g of absolute alcohol/year. A low discordance i.e. less than 4 000 g, was found in 13 pairs (19 %). All pairs were classified in an identical manner in 1967 and 1972/73 with respect to the intra pair high-low relationship.

Comments

Repeatability on the basis of the intra-pair high-low relationship thus amounted to 100 %. The reduced discordance in 1972/73 as compared to 1967 may have been due to e.g. incongruities in the measurement technique. Furthermore there is almost always regression towards the mean in studies of this type (Remington & Schork 1970). There may also have been true changes in drinking habits from 1967 to 1972/73. Fairly constant drinking patterns have been reported by Jellinek (1960) and Nilsson (1973) for middle-aged men, but a decrease in consumption has been shown for older subjects (Jellinek 1960; Carlsson 1970). In any case, data point to a consistent discordance in volumes consumed in the past 6 year period.

A average alcohol consumption for Swedish adult 19 1 said to amount to 4 500 g of absolute alcohol/year (Rapport 19 3).

TABLE 4 *Intra-pair discordance expressed in grams of absolute alcohol according to the 1967 questionnaire and the 1972/73 interview*

	Discordance in grams of abs. alc./yes	MZ (N=14 pairs)		DZ (N=36 pairs)		MZ+DZ (N=70 pairs)	
		> 8000	4000-8000	> 8000	4000-8000	> 8000	4000-8000
1972/73	> 8000	7	1	30	4	37	5
	4000-8000	2	—	12	1	14	1
	< 4000	4	—	9	—	13	—

SOME ANAMNESTIC VARIABLES

One method of determining a subject's alcohol habits is to identify certain behavior related to high consumption. Blackouts and the use of eye openers ("literkillare") are some of the variables often used in defining alcoholism (Jellinek 1960, Carlsson 1970). These variables as well as the following were elucidated in the 1972/73 interview: drinking behaviour at parties, intoxication, hangover and "more than half a bottle of hard liquor or a bottle of wine on the same occasion, alcohol problems, violence when drunk, drunken driving, police contacts when drunk, neglected work or marriage problems due to alcohol. The last six variables are not presented because of their low prevalence. The remaining anamnestic variables are presented in Table 5.

Blackouts

A larger number of blackouts was found among the high alcohol consumers in the pooled zygosity group (MZ+DZ). Seventeen subjects in the high alcohol group (HAG) versus six in the low alcohol group (LAG) reported blackouts ($p < 0.01$). Three pairs were concordant as to this criterion.

Eyeopeners

A positive answer to the question on the use of eyeopeners was significantly more common among the HAG than the pooled zygosity group ($p < 0.05$). Eleven twins in this group gave a positive reply versus 3 subjects in the LAG.

Comments

Blackouts and the use of "eyeopeners" are two of the variables commonly associated with a high

alcohol consumption and alcoholism (Jellinek 1960, Carlsson 1970). Blackouts may be "one of the best indicators of incipient alcoholism" (Wallgren & Barry III 1970).

Drinking behavior at parties

Four glasses of hard liquor or more at parties were considered heavy consumption. Thirty five HAG subjects versus 11 LAG subjects in the pooled zygosity group submitted positive replies on this criterion ($p < 0.001$).

More than half a bottle on the same occasion

Answers were considered positive when subjects reported drinking more than half a bottle of hard liquor or more than a bottle of wine on the same occasion at least once a month. In the pooled zygosity group 22 HAG subjects versus 4 LAG subjects gave a positive answer to this variable ($p < 0.001$).

Intoxication and hangover

In the pooled zygosity group, 70 HAG subjects versus 11 LAG subjects admitted alcohol intoxication at least once a month ($p < 0.05$). Hangover was somewhat more prevalent among the high alcohol consumers, but this finding was not significant.

Comments

During the successive development of alcohol dependence intoxication becomes an increasingly urgent desire and usually an increasingly prevalent condition (Wallgren & Barry III 1970). The same authors were less certain that a hangover is a sign of alcohol dependence. In addition, it seems

TABLE 5 Frequency of concordance and discordance with respect to anamnestic information in relation to alcohol consumption

		LOW ALC								
		MZ			DZ			MZ+DZ		
		pos	neg	tot	pos	neg	tot	pos	neg	tot
Blackouts	pos	1	2	3	2	12*	14	3	14**	17
	neg	0	11	11	3	39	42	3	50	53
	tot	1	13	14	5	51	56	6	64	70
Eyeopeners	pos	0	0	0	1	10*	11	1	10*	11
	neg	0	14	14	2	43	45	2	57	59
	tot	0	14	14	3	53	56	3	67	70
Heavy consumption at parties	pos	1	4	5	5	25***	30	6	29***	35
	neg	1	8	9	4	22	26	5	30	35
	tot	2	12	14	9	47	56	11	59	70
HIGH ALC										
More than a half-bottle of alcohol	pos	0	2	2	2	18***	20	2	20***	22
	neg	0	12	12	2	34	36	2	46	48
	tot	0	14	14	4	52	56	4	66	70
Intoxication	pos	0	2	2	4	14	18	4	16*	20
	neg	0	12	12	7	31	38	7	43	50
	tot	0	14	14	11	45	56	11	59	70
Hangover	pos	0	1	1	3	9	12	3	10	13
	neg	1	12	13	7	37	44	8	49	57
	tot	1	13	14	10	46	56	11	59	70

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

TABLE 6.5 *Subject classification of alcohol habits*

Grade Habit	MZ		DZ		MZ+DZ	
	High alc.	Low alc.	High alc.	Low alc.	High alc.	Low alc.
1 Total abstinence	0	2	0	3	0	5
2 Occasional drinking	1	7	3	18	4	25
3 Moderate drinking	13	5	29	53	42	38
4 Occasionally drunk						
often drunk-alcoholic	0	0	20	2	20	2
Undecided	0	0	4	0	4	0
Total	14	14	56	56	70	70

reasonable to assume that subjects less frequently exposed to intoxication and hangover are more likely to recall and report rare incidents. On the other hand subjects more commonly exposed to these conditions may have a tendency to ignore both intoxication and hangover and neglect to report on these items.

Subjective classification of alcohol habits

In the 1972/73 investigation twins were asked to describe their personal alcohol habits using one alternative on a graded scale. The results are given in Table 6. In the pooled zygosity group 30 (43 %) out of the 70 low consumers reported total abstinence or occasional drinking. The corresponding figure for high consumers was 4 (6 %). As far as moderate drinking was concerned, there was almost an equal number of subjects in the LAG and HAG i.e. 38 (34 %) and 42 (60 %) subjects respectively. The alternatives occasionally drunk — often drunk — alcoholic were chosen by 20 HAG subjects (29 %) versus 2 LAG subjects (3 %). The differences with respect to subjective classification referred to DZ twins but MZ twins also showed obvious disparities.

Comment

The extreme alternatives apparently provided discrimination of the two groups. There were 20 concordant twin pairs among subjects who chose the alternative "moderate drinking". Fourteen of

these 20 pairs were found to display an alcohol discordance exceeding 4 000 g of absolute alcohol/year. It is concluded that the subjective rating certainly supports the intra pair relationship obtained from the calculation of alcohol but shows that calculated discordance expressed in grams of absolute alcohol/year is a far more efficient measure. In addition, the only subject classifying himself as being an alcoholic belonged to the high alcohol group.

PHYSICAL FINDINGS ASSOCIATED WITH HIGH ALCOHOL CONSUMPTION

The following physical findings often associated with a high alcohol intake (Sherlock 1968, Carlsson 1970, Wallgren & Barry III 1970) were analysed: hepatomegaly, tremor (coarse, confirmed), vascular spiders — paper money skin, Dupuytren's contracture, jaundice, erythema of the palms, acrotes, gynecomastia and scar following stomach surgery. The last four variables are not presented due to low prevalence. The results of the examination are shown in Table 7.

Hepatomegaly

Hepatomegaly was one of the somatic findings which distinguished between the high and low consumers of alcohol. Twenty-two (31 %) of the high consumers in the pooled zygosity group were judged to have an enlarged liver as compared to 5 (7 %) of the low consumers ($p < 0.001$). Three pairs were concordant as to this criterion.

TABLE 7 Frequency of concordance and discordance in respect to one physical finding in relation to alcohol as imple

		LOW ALC								
		MZ			DZ			MZ+DZ		
		pos	neg	tot	pos	neg	tot	pos	neg	tot
Hepatomegaly	pos	0	3	3	3	16**	19	3	19***	22
	neg	0	11	11	2	35	37	2	46	48
	tot	0	14	14	5	51	56	5	65	70
Tremor	pos	0	2	2	1	19**	20	1	21**	22
	neg	1	11	12	5	31	36	6	42	48
	tot	1	13	14	6	50	56	7	63	70
HIGH ALC Spiders - paper money* skin	pos	0	2	2	1	12*	13	1	14**	15
	neg	0	12	12	3	40	43	3	52	55
	tot	0	14	14	4	52	56	4	66	70
Jaundice	pos	0	1	1	0	5	5	0	6	6
	neg	0	13	13	1	50	51	1	63	64
	tot	0	14	14	1	55	56	1	69	70
Dupuytren's contracture	pos	1	2	3	1	10**	11	2	12*	14
	neg	2	9	11	1	44	45	3	53	56
	tot	3	11	14	2	54	56	5	65	70

* = $p < 0.05$

** = $p < 0.01$

*** = $p < 0.001$

Comments

Fatty infiltration of the liver causing hepatomegaly as a complication of high alcohol intake, is well known (Sherlock 1968 Wallgren & Barry III 1970 Carlsson 1970). Even portal cirrhosis is often manifested as hepatomegaly (Sherlock 1968). Wrobe *et al.* (1971) was able to disclose triglyceride accumulation in the liver of apparently normal subjects after single dose of alcohol.

Tremor

Tremor was significantly more common in the HAG. In the pooled xygosity group, 22 of the high consumers as opposed to 7 of the low consumers were judged to have a coarse tremor ($p < 0.01$).

Comments

Tremor is a variable phenomenon, partly under

TABLE 8 *Serum enzymes in relation to alcohol consumption.*

	MZ			DZ			MZ+DZ		
	High alc.	Low alc.	No of pairs	High alc.	Low alc.	No of pairs	High alc.	Low alc.	No of pairs
	M(SD)			M(SD)			M(SD)		
SGOT	19.3(5.4)	18.7(4.6)	14	23.4(8.0)	19.4(4.2)	52	2.6(7.7)	19.2(4.3)**	66
SGPT	13.7(3.7)	16.3(8.0)	14	19.8(10.7)	18.0(8.9)	52	18.6(10.0)	17.6(8.7)	66
SALP	20.5(9.9)	11.1(6.6)	14	23.8(8.6)	21.7(7.6)	51	23.1(8.9)	20.8(7.5)	65

* = $p < 0.05$

** = $p < 0.001$

direct control from cerebral centers and therefore subject to the influence of mood and conscious effort. A positive relationship between finger tremor and alcohol consumption has been described by Carne (1965) Carlsson (1970) and Wallgren & Barry III (1970).

Vascular spiders and paper money skin

The prevalence of vascular spiders and "paper money skin" was significantly higher in the HAG than in the LAG. These findings were noted in 15 of the high alcohol consumers as opposed to 4 of the low alcohol consumers ($p < 0.01$).

Comments

Arterial spiders and "paper money skin" may appear in cases of deranged hepatic function, and the appearance of fresh spiders is indicative of progressive liver damage. According to Sherlock (1968) spiders are especially florid in the alcoholic.

Jaundice and Dupuytren's contracture

None of the twins in the subject group displayed any signs of confirmed jaundice but suspected jaundice was generally found more frequently in the high alcohol group. No significant inter group difference was noted in respect of this finding. Dupuytren's contracture, suspected or confirmed, was more prevalent in the HAG of the pooled zygosity group ($p < 0.05$). In this group 14 of the high consumers as opposed to 5 of the low consumers were judged to have suspected or confirmed contracture ($p < 0.05$).

Comments

Jaundice is a sign of impaired bilirubin metabolism and may be caused by many different mechanisms, one being a high intake of alcohol (Sherlock 1968). Mild forms of jaundice are difficult to diagnose clinically and laboratory tests are necessary to confirm the diagnosis. These were not judged to be of value in the present investigation. According to Sherlock (1968) Dupuytren's contracture is sometimes a characteristic of cirrhosis in alcoholics. Other authors (Cochrane 1972, Benjafield & Rutter 1972) have stressed an autosomal dominant inheritance in this condition and that it may be linked to chronic alcoholism.

SERUM ENZYME PATTERNS

Serum aspartate aminotransferase (SGOT), serum alanine aminotransferase (SGPT) and serum alkaline phosphatase (SALP) have long been used as diagnostic aids in alcoholism and diseases of the liver (Sherlock 1968). Thus the twin pairs were analysed with respect to these aforementioned serum enzymes. The results are shown in Table 8. The twins discordant at > 1000 g of absolute alcohol/month were also analysed with respect to serum gamma-glutamyl transpeptidase (SGT). Some of the twin pairs were not included in the data on serum enzymes, in all cases but one owing to lost samples (3 samples for SGOT and SGPT, 4 samples for SALP). This means that in whole twin was "lost" if the value of one member of a pair was lost. One pair was excluded as the low alcohol consumer long since a teetotaler had recently diagnosed liver disease following treatment

with chlorpromazine. This effect has been discussed by Sherlock (1968)

SGOT

Significant differences were found between the high and low alcohol groups with respect to SGOT. Mean values in the pooled zygosity group were 22.6 and 19.2 U/l in the HAG and LAG respectively ($p < 0.001$)

Comments

This intra-pair comparison thus revealed a significant disparity with regard to SGOT. Both groups, however, fell within normal limits. The SGOT values suggest that moderate alcohol discordance need not necessarily give rise to any serious liver damage. Bang *et al* (1938) found a rise in SGOT after acute alcohol intoxication in 27 of 35 alcoholic patients. Broholt *et al* (1966) was able to show that a single dose of alcohol, corresponding to 3 g/kg body weight, administered to apparently healthy men produced a significant rise in SGOT for a few days after alcohol intake.

SGPT

No obvious differences were obtained in the pooled zygosity group with respect to SGPT

Comments

Broholt *et al* (1966) found that the SGOT values were higher than SGPT values after a large dose of alcohol given to apparently healthy men. According to Wallgren & Barry III (1970) habitual drinkers have elevated SGOT, SGPT and SALP values, while SGPT and SGT in particular are reported to be elevated in alcoholics studied during institutional treatment.

SALP SGT

Significant differences were obtained ($p < 0.05$) with regard to SALP. Mean values in the pooled zygosity group amounted to 3.1 and 20.8 U/l in the HAG and LAG respectively.

The greatest disparities between mean values were obtained for the twin pairs discordant with respect to $> 1,000$ g of absolute alcohol/month

analysed for SGT. This analysis, which has been presented elsewhere (Myrthed & Bergström 1973) disclosed mean values of 33 and 11 U/l for the HAG and LAG respectively ($p < 0.01$)

Comments

Differences between mean values for SALP were thus not pronounced, but intra pair testing revealed significant disparities between high and low consumers. As in the case of SGOT the results support alcohol discordance but refute the presence of any serious liver damage in the alcohol positive group. Elevated SALP values have been reported in alcoholics (Wallgren & Barry III 1970). Analysis of SGT in the highly discordant mentioned group produced the most striking disparities. SGT has been termed a sensitive and specific test in subjects regarded as heavy drinkers rather than alcoholics in the determination of liver cell damage in the absence of clinical evidence of liver disease (Rosalki & Riu 1972, Rosalki 1973).

DISCUSSION

The difficulties encountered in obtaining an adequate estimate of alcohol consumption have been discussed by e.g. Kalj (1960), Carlsson (1970) and Björkman (1971). A tendency to underestimate this has been found in comparisons of subjective answers with objective measurements of volumes sold. Björkman (1971) underlined the advantage of mailed questionnaires compared to personal interviews in detecting extremes of consumption. In the present study data on alcohol consumption were not obtained in personal interviews. The respondents answered the alcohol questions by themselves. Their replies were subsequently checked in interviews. This procedure was felt to increase the validity of the answers.

In any study concerning the effects of alcohol on somatic status, life-long consumption would probably provide the best prerequisite for drawing any conclusions. On the other hand, fairly constant drinking behaviour has been reported by Jellinek (1960) and Nilsson (1973) in middle aged men. Information on alcohol intake in the present

study was available for two periods. The complete repeatability with respect to the intra pair high-low relationship points to longstanding alcohol discordance for the twin pairs selected.

As far as specific anamnestic variables were concerned, significant disparities were found between high and low alcohol consumers with regard to the following: blackouts, "eyecopers", intoxication heavy consumption at parties, "more than half a bottle on the same occasion" and subjective classification of personal habits. These positive findings further supported the selection's validity. On the other hand, no differences could be shown in "subjective alcohol problems, violence when drunk, drunken driving, in trouble with the police when drunk, neglecting work, or marriage problems due to alcohol. As to these last variables, a great deal of bias may be expected, as subjects probably responded in accordance with social desirability. Several of the anamnestic variables included are associated with alcoholism. On the other hand, the impression was that many of the high consumers could not really be classified as alcoholics. This might be ascribable to the fact that few questionnaires were probably mailed back by alcoholics in 1967 and to the high concordance regarding consumption of large volumes of alcohol (Kaij 1960, Partanen *et al* 1966, Jonsson & Nilsson 1968). Thus, such pairs could not have been called in for examination in 1972/73. Furthermore the strongest significances were consistently obtained for variables pertaining to alcohol volumes consumed (consumption at parties, subjective classification of personal habits, "more than half a bottle on the same occasion") but not for variables commonly considered indicative of alcoholism (blackouts, eyecopers, intoxication).

As far as physical findings associated with heavy alcohol intake were concerned, a significantly greater proportion of the high consumers were found to have hepatomegaly tremor vascular spiders, "paper money skin and Dupuytren's contracture. Findings indicative of serious liver damage such as patent or suspected jaundice, ascites, and gynecomastia, were not more prevalent

TABLE 9 Distribution of twin pairs by alcohol discordance class according to the 1972/73 interview

Class	Discordance in grams abs alc./year	MZ	DZ	MZ+DZ	Age MZ+DZ M(SD)
A	> 10,000	5	23	30	34.8 (3.4)
B	3,000—10,000	5	18	23	33.9 (3.9)
C	< 3,000	4	13	17	35.2 (3.0)
Total		14	56	70	34.6 (4.8)

in the high alcohol group. Results of young moderate intra pair alcohol discordance were also found with respect to serum enzymes. Although a highly significant difference was noted for SGOT only 2 of the high consumers displayed serum values exceeding 35 U/l, indicating that liver damage would scarcely have been suspected in any of the alcohol consumers in an ordinary medical examination. Similar results were found with respect to SGPT and SALP. The moderate alcohol discordance, is also underlined by the significantly higher SALP and SGT values found among the alcohol positive twins. It is concluded that the results in terms of specific anamnestic variables, physical findings and serum enzymes are in favour of a subject group consisting of moderately alcohol discordant twin pairs and that the data permit the assignment of the twins into a high or a low alcohol group.

GRADING OF ALCOHOL DISCORDANCE

The initial selection of the twin pairs was made on basis of Twin Registry data in accordance with the 1967 questionnaire. Since 6 years had elapsed since the 1967 questionnaire the information in the 1972/73 interviews was regarded as the most adequate basis for discordance classification at the time of the investigation. Thus, this measurement was used for calculation of intra pair differences, and the twins were divided into 3 different discordance classes (Table 9)

CLASS A discordance > 10,000 grams/year

CLASS B discordance 3,000—10,000 grams/year

CLASS C discordance < 3,000 grams/year

SUMMARY

Seventy tentatively alcohol discordant twin pairs were investigated in order to determine the validity of discordance classification. One hundred per cent repeatability was found between the 1967 and the 1972/73 classification with regard to the intra-pair high-low relationship. Significant differences were obtained between the high and low alcohol group as to the prevalence of the following anamnestic variables: Blackouts, eyeopeners, intoxication, heavy consumption at parties, more than half a bottle on the same occasion and subjective

classification of personal alcohol habits. In addition, the alcohol positive group showed a significantly increased prevalence of the following physical findings: Hepatomegaly, tremor, vascular spiders, paper money skin and Dupuytren's contracture. Serum enzymes showed significant disparities in SGOT, SALP and SGT values, the higher values occurring among high alcohol consumers. It is concluded that the alcohol discordance obtained is valid and permits a separation of twins into a high alcohol group (HAG) and a corresponding low alcohol group (LAG).

Smoking and some pulmonary symptoms

Various epidemiological investigations have shown that morbidity and mortality due to IHD are more prevalent in smokers than in non-smokers. It is assumed that the variables associated with IHD e.g. smoking, may be explained in part by alcohol consumption, as smokers are known to consume more alcohol than non-smokers. The purpose of the work described in this chapter was to determine the extent to which the association between alcohol consumption and cigarette smoking persists when the influence of genetic and early environmental factors is assumed to be minimal. A number of pulmonary symptoms were also studied.

PREVIOUS STUDIES

Smoking and IHD

A possible link between IHD and cigarette smoking was noted in the report of the Royal College of Physicians in 1962. Two years later the U.S. Surgeon-General concluded that male cigarette smokers have a higher death rate from coronary artery disease than non smoking males, but it is less clear that the association has causal significance (U.S. Public Health Service 1964). Reports on the relationship between smoking and IHD have been continuously renewed ever since, in "The Health Consequences of Smoking" published in 1968 and 1971. These reports concluded that cigarette smokers, particularly men and those in young age groups, were found to have a higher mortality from IHD than non-smokers. The association was found to be stronger for sudden death and fatal myocardial infarction and less definite for angina pectoris.

The statement in the report of the Royal College of Physicians (1971) "that cigarette smoking is an important factor in causing coronary heart disease and that the general avoidance of cigarettes would probably diminish the number of deaths

in this condition is a hypothesis that has yet to be proved. That cigarette smoking aggravates the effect of other coronary risk factors in predisposing to IHD has also been postulated (The National Heart Foundation of New Zealand 1971).

Reviews concerning IHD and associated risk factors have also covered the question of smoking (Epstein 1965, Stamler 1967, 1973, Semborg 1970, Fejfar 1972, Hay 1977). Prospective studies have disclosed an association between smoking and IHD (Kannel *et al.* 1968, Carlson & Böttiger 1972, Wilhelmsen *et al.* 1973). In the Framingham prospective study (Kannel *et al.* 1968) the risk of IHD developing in men was proportional to the number of cigarettes smoked/day but unrelated to the duration of the habit. In a nine year follow-up of 3168 men in the Stockholm prospective study the incidence of new IHD episodes was found to be strongly related to smoking (Carlson & Böttiger 1972). Wilhelmsen *et al.* (1973) using a multiple logistic model, found that smoking was one of the three most powerful predictors of IHD.

Yet the data on morbidity and mortality with respect to IHD in smoking-discordant twin pairs can not be ignored. Lundman (1966) who carried out a clinical investigation of about 200 pairs of smoking-discordant twins failed to find any difference with respect to any IHD manifestations. Neither serum cholesterol nor blood pressure was higher in the smokers than in the non-smoking co-twins. In fact an inverse relationship was found. Liljefors (1970) studying IHD-discordant twin pairs could not demonstrate that the amount of cigarettes smoked differentiated between twins who probably had IHD and those who probably had not.

In a questionnaire study based on the Swedish Twin Registry Cederlöf *et al.* (1966) found an

increased morbidity from angina pectoris among male smokers when the investigational material was regarded as a population sample. However no corresponding increased morbidity could be established when intra-pair comparisons were made between smokers and non-smokers. In continuous investigations of the Swedish Twin Registry (Friberg *et al* 1970 Friberg *et al* 1973) for mortality in twin pairs differing with respect to smoking, an association was found between smoking and mortality including IHD-death, in the DZ but not in the MZ group.

The high incidence of IHD in smokers has drawn attention to the pharmacological effects of acute and prolonged smoking. Lundman (1966) and Rose (1973) have discussed the acute effects of nicotine. By catecholamine release, nicotine has been found to cause an increase in heart rate, conduction rate, stroke volume myocardial irritability and a slight rise in blood pressure and in levels of free fatty acids. Moreover the acute and chronic effects of carbon monoxide have become the subject of increasing interest (U.S. Surgeon-General's Report 1968, 1971 Hay 1972, Rose 1973 Wald *et al* 1973 Aronow 1973). Carbon monoxide reduces the oxygen content of capillary blood and interferes with oxygen release to the myocardium. Astrup (1969 1973) drew attention to the possible atherogenic role of carbon monoxide. An increased endothelial permeability to lipoproteins leading to subendothelial oedema has been shown in animals exposed to high levels of carbon monoxide (Astrup 1969 1973 Kjeldsen *et al* 1972).

Pulmonary findings and symptoms in relation to IHD

An indication that a reduced vital capacity is associated with IHD was initially found in the Framingham Prospective Study (Hannel *et al* 1968). This relationship persisted when the material was subdivided into three classes according to the number of cigarettes smoked. In a 5-year follow-up study of 7,982 men aged 40–59 and initially free from demonstrable IHD Keys *et al* (1972 a) found that low values for vital capacity were associated with an increased risk of

developing IHD. However the significant relationship disappeared when comparisons were made age-specific using 5 year classes, but still there was a tendency for a higher rate of IHD development in the quintiles with the lowest vital capacity.

Using a multiple logistic model Wilhelmsen *et al* (1973) found that dyspnoea, defined according to WHO criteria significantly increased the risk of IHD. It was suggested that the link between exertional dyspnoea and IHD might indicate that subjects complaining of dyspnoea already suffer from some form of cardiovascular or pulmonary dysfunction.

Smoking in relation to heredity

Twin studies by Fisher (1958) and Friberg *et al* (1959) have shown that smoking habits are more similar in monozygotic than in dizygotic twins. These results were confirmed by Cederlöf (1966) who reported concordance with respect to smoking in 57% of MZ and 50% of DZ twin pairs. The corresponding figures for concordant non smoking pairs were 23 and 19% respectively. Smoking thus seems to be influenced by genetic factors, although the habit may be regarded as an environmental trait linked to the genetic constitution (Liljefors 1970).

Smoking and alcohol

Smokers are known to consume more alcohol than non smokers (Higgins *et al* 1967 Higgins & Kjeldsberg 1967 Carlsson 1970). In subjects with lung cancer from cigarette smoking, high correlation was also found between smoking and alcohol consumption (Burch & de Pasquale 1967). A comparison of 321 lifelong non-smokers and 279 continuing and former cigarettes smokers in consecutive classes of medical students at Johns Hopkins, disclosed significant differences in levels of coffee and alcohol consumption (Thomas 1973). Friberg *et al* (1973) was able to demonstrate that up to 10% of non-smoking twins were registered in a nationwide alcohol registry as opposed to 30% of the smokers consuming more than 10 cigarettes/day. It has been suggested that part of the reported increased mortality among smokers

TABLE 10 *Smoking status in relation to alcohol consumption*

	MZ (N=14 pairs)		DZ (N=36 pairs)		MZ+DZ (N=70 pairs)			
	High alc.	Low alc.	High alc.	Low alc.	High alc.	%	Low alc.	%
Non smokers	0	2	6	11	6	(8.6)	13	(18.6)
Former smokers	5	8	13	8	18	(25.7)	16	(22.9)
Present smokers	9	4	37	37	46	(65.7)	41	(58.5)

is due to associated factors, such as alcohol consumption, rather than to smoking by itself (Ceder 18f 1966 Friberg *et al* 1973)

MATERIAL AND METHODS

The subject group is described in chapter I. The twins were interviewed with respect to smoking habits and classified as follows.

Non smokers were defined as subjects who had never smoked any form of tobacco.

A former smoker was defined as a subject who denied regular smoking at the time of the investigation but had previously been regular smoker.

A present smoker was defined as a subject who smoked regularly at the time of the investigation.

A gas/tt smoker was defined as a person who smoked cigarettes exclusively.

A mixed smoker was defined as a person who smoked both cigarettes, cigars or a pipe.

A cigar/pipe smoker was defined as a subject who smoked cigars or pipe exclusively.

The cigarette smokers were separated into different groups depending upon the number of cigarettes consumed. 1-4, 5-14, 15-24 and ≥ 25 cigarettes/day. In the quantitative evaluation of the intra-pair smoking habits this grouping was used to identify the twin showing the highest cigarette consumption.

Pulmonary symptoms were defined as described in Methods (p. 18).

RESULTS

Smoking

Smoking status

A description of the smoking status of the subject group is given in Table 10. All but 6 of the high alcohol consumers (HAG) in the pooled zygosity group had smoked at some time in their lives, i.e. 64 subjects (91.4 %). Present smoking was reported by 46 subjects (65.7 %) in the HAG. In the low alcohol group (LAG) there were 13 (18.6 %) non-smokers, i.e. twice as many as in the HAG. A total of 57 subjects (81.4 %) in the LAG reported smoking during some period of their lives, 41 of whom (58.5 %) were present smokers. It was apparent that the two groups did not differ markedly with regard to smoking, as shown in an intra-pair comparison. (Table 14).

Type of smoking

A preference for smoking cigarettes was the predominant type of smoking found in the HAG as opposed to cigars or a pipe in the LAG (Table 11). Six HAG subjects (8.6 %) smoked cigars and/or pipe exclusively while the corresponding number for the LAG was 21 subjects (30.0 %). This difference is statistically significant (p

TABLE 11 *Type of smoking (former and present smokers combined) in relation to alcohol consumption.*

	MZ (N=14 pairs)		DZ (N=36 pairs)		MZ+DZ (N=70 pairs)			
	High alc.	Low alc.	High alc.	Low alc.	High alc.	%	Low alc.	%
Cigarette smokers	6	3	25	17	31	(44.3)	20	(28.6)
Mixed smokers	4	4	23	12	27	(38.6)	16	(22.9)
Cigar/pipe smokers	4	3	2	16	6	(8.6)	21	(30.0)

TABLE 12 Number of cigarettes smoked (including former smokers) in relation to alcohol consumption

	MZ (N=14 pairs)		DZ (N=56 pairs)		MZ+DZ (N=70 pairs)	
	High alc.	Low alc.	High alc.	Low alc.	High alc. %	Low alc. %
Cigarettes only						
1-4	1	1	2	6	3 (4.3)	7 (10.0)
5-14	2	1	3	3	7 (10.0)	6 (8.6)
15-24	2	1	12	5	14 (20.0)	6 (8.6)
25-	1	0	6	1	7 (10.0)	1 (1.4)
Cigarettes mixed						
1-4	1	1	7	1	8 (11.4)	2 (2.9)
5-14	0	2	11	7	11 (15.7)	9 (12.9)
15-24	3	1	4	4	7 (10.0)	5 (7.2)
25-	0	0	1	0	1 (1.4)	0 (0.0)

<0.001) Mixed smoking was reported by 27 HAG subjects (38.6%) and by 16 LAG subjects (22.9%). Thirty-one (44.3%) and 20 (28.6%) high and low consumers respectively smoked cigarettes only.

Quantity smoked

Table 12, which includes present and former smokers, shows that a high alcohol consumption is also accompanied by a high cigarette consumption. 67.1% of the HAG reported ≥ 5 cigarettes/day and 38.7% of the LAG. The corresponding figures for heavy cigarette consumption (≥ 25 /day) were 11.4% and 1.4% respectively.

The results with respect to present cigarette smoking are found in Table 13. Despite a reduced number of smokers in both groups, the association between high alcohol intake and a high level of cigarette consumption was repeated.

When the number of cigarettes smoked daily was analysed by means of intra-pair comparison, pronounced disparities were also found between high and low alcohol consumers (Table 14). When former smokers were included in the pooled zygosity group 42 HAG subjects displayed a higher level of cigarette consumption than their co-twins. The corresponding number in the LAG was 9 ($p < 0.001$). An estimation of present cigarette

TABLE 13 Number of cigarettes smoked (present smokers) in relation to alcohol consumption

	MZ (N=14 pairs)		DZ (N=56 pairs)		MZ+DZ (N=70 pairs)	
	High alc.	Low alc.	High alc.	Low alc.	High alc. %	Low alc. %
Cigarettes only						
1-4	1	0	2	3	3 (4.3)	3 (7.2)
5-14		1	3	4	7 (10.0)	5 (7.2)
15-24	0	1	8	1	8 (11.4)	5 (7.2)
25-	1	0	6	1	7 (10.0)	1 (1.4)
Cigarettes, mixed						
1-4	0	0	3	1	3 (7.2)	1 (1.4)
5-14		0	6	3	6 (8.6)	5 (7.2)
15-24		1	3	3	5 (7.2)	4 (5.7)
25-		0	0	0	0 (0.0)	0 (0.0)

TABLE 14 Frequency of concordance and discordance in the respect to smoking in relation to alcohol consumption

		LOW ALC								
		MZ			DZ			MZ+DZ		
		pos	neg	tot	pos	neg	tot	pos	neg	tot
HIGH ALC	Ever smoked (all types of smoking)	pos 12 2 14	neg 0 0 0	tot 12 2 14	pos 41 9 50	neg 4 2 6	tot 45 11 56	pos 53 11 64	neg 4 2 6	tot 57 13 70
	Present smokers (all types of smoking)	pos 3 6 9	neg 1 4 5	tot 4 10 14	pos 25 12 37	neg 12 7 19	tot 37 19 56	pos 28 18 46	neg 13 11 24	tot 41 29 70
	Cigarette level (including former smokers)	pos 4 5 9	neg 2 3 5	tot 6 8 14	pos 8 37*** 45	neg 7 4 11	tot 15 41 56	pos 12 42*** 54	neg 9 7 16	tot 21 49 70
	Cigarette level (present smokers)	pos 0 6 6	neg 2 6 8	tot 2 12 14	pos 7 25* 32	neg 12 12 24	tot 19 37 56	pos 7 31** 38	neg 14 18 32	tot 21 49 70

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

consumption gave the same results although less pronounced ($p < 0.01$)

Pulmonary symptoms

Pulmonary symptoms are presented in Table 15. In the pooled zygosity group, cough, defined as regular morning cough, was found in 23 HAG and 10 LAG subjects respectively ($p < 0.01$).

Phlegm was reported by 30 twins in the HAG as opposed to 18 twins in the LAG ($p < 0.05$).

Eight subjects in the HAG had dyspnea defined as grades 3–5 (Grade 3 = shortness of breath when walking with other people of own age on level ground). One subject in the LAG was positive according to this definition ($p < 0.05$). One

of the MZ and 2 of the DZ pairs could not be assessed, as one of the twins had decreased myoskeletal locomotive power.

Bronchial asthma was found in 26 of the HAG as opposed to 14 of the LAG ($p < 0.01$).

DISCUSSION

Small disparities were found between high and low alcohol consumers with regard to smoking. However, more than twice the number of subjects who never had smoked were found in the low alcohol group. On the other hand, the intra-pair comparison showed a stronger association between the number of cigarettes smoked and the amount of alcohol consumed than the results of the evalua-

TABLE 15 Frequency of on oral and dis cordant with respect to the pulmonary symptom / class to alcohol consumption

		LOW ALC								
		MZ			DZ			MZ+DZ		
		pos	neg	tot	pos	neg	tot	pos	neg	tot
Cough	pos	0	4	4	6	13*	19	6	17**	23
	neg	1	9	10	3	34	37	4	43	47
	tot	1	13	14	9	47	56	10	60	70
Phlegm	pos	1	3	4	10	16*	26	11	19*	30
	neg	2	8	10	5	25	30	7	33	40
	tot	3	11	14	15	41	56	18	52	70
HIGH ALC										
Dyspnea 1)	pos	0	0	0	0	8*	8	0	8*	8
	neg	0	13	13	1	45	46	1	58	59
	tot	0	13	13	1	53	54	1	66	67
Bronchial asthma	pos	1	2	3	8	15*	23	9	17**	26
	neg	0	11	11	5	28	33	5	39	44
	tot	1	13	14	13	43	56	14	56	70

1) neg = stages 1-2
pos = stages 3-5

* = $p < 0.05$
** = $p < 0.01$

tion of the smoker/non-smoker dichotomy. This was true for MZ and DZ groups. Highly significant differences were found between high and low alcohol subjects in the pooled zygosity group (MZ+DZ) with respect to number of cigarettes smoked when former smoking were included. Similar results were obtained with regard to the present level of cigarette consumption, although less pronounced. That heavy smoking is associated with a high alcohol intake was previously reported by e.g. Higgins *et al* (1967) and Thomas (1973). In a study of non-smokers and two groups of heavy smokers, Thomas found that coffee in-

take and alcohol frequency contributed most heavily to the discrimination. When non-smokers were compared with two groups of light smokers, on the other hand, alcohol frequency did not produce any significant discrimination. The importance of the level of cigarette consumption to the magnitude of association was also underlined by Friberg *et al* (1973).

As to type of smoking, a larger number of pipe and cigar smokers were found in the alcohol negative group among DZ twins. This would appear to indicate that low consumers of alcohol prefer other types of tobacco than cigarettes. The

association of high alcohol intake in combination with large amounts of cigarettes consumed, as opposed to moderate alcohol consumption and cigar or pipe smoking may be a reflection of different personality types. In this context it is interesting to note that reports by the U.S. Surgeon-General (1968, 1971) Kannel *et al* (1968) and Fejfar (1972) have failed to reveal any link between the smoking of pipes or cigars and IHD morbidity and mortality.

An increased prevalence of pulmonary symptoms, such as cough, phlegm, dyspnea and asthma was found in the alcohol-positive group. The results were mainly derived from DZ twins but were consistent with results from the MZ group. Significantly higher prevalences were noted for all types of symptoms in the DZ and pooled zygosity group. The disparity might have been caused by alcohol, which has been reported to act as a specific risk factor in chronic pulmonary disease owing to the effect of alcohol on mechanisms responsible for the removal of bacteria and inert particles from the lungs (Green & Kass 1965). However a more probable explanation is that the pulmonary symptoms are caused by heavier smoking. Lundman (1966) investigating smoking-discordant twin

pairs, found a significantly higher prevalence of respiratory symptoms, such as morning cough, chronic cough and morning phlegm, in the smokers.

SUMMARY

Seventy alcohol discordant twin pairs were investigated with respect to smoking habits and some pulmonary symptoms. Smoking was found to be slightly more prevalent in the high alcohol group. Pipe or cigar smoking turned out to be significantly more frequent among low consumers of alcohol. The numbers of cigarettes smoked showed the most striking differences. A higher level of cigarette smoking was noted in the alcohol positive group. The difference was significant both for present smokers and when former smokers were included. Pulmonary symptoms, such as cough, phlegm, dyspnea and bronchial asthma were more frequently found in subjects with a high alcohol consumption. Significant disparities were found in the prevalence of all these symptoms. It is concluded that moderate longstanding alcohol consumption is accompanied by a high level of cigarette smoking and by the appearance of pulmonary symptoms, even when the influence of genetic and early environmental factors is minimal.

Blood pressure

While the association between hypertension and IHD has been well established, the extent to which this link may be ascribable to external factors is not fully known. It is supposed that variables associated with IHD such as blood pressure, may be due in part to a high consumption of alcohol. An elevated blood pressure has been found in groups characterized by high alcohol consumption. The purpose of this chapter is to report on the connection between alcohol consumption and blood pressure when the influence of genetic and early environmental factors is assumed to be minimal.

PREVIOUS STUDIES

Blood pressure in relation to IHD

The induction of atherosclerosis experimentally in animals by a high intake of lipids appears to be accelerated by hypertension (Walkerlin *et al* 1957 Heptinstall & Porter 1957). Retrospective studies of humans have revealed a relationship between IHD and hypertension. In such studies, reviewed by Gofman (1959) the incidence of previously occurring hypertension was determined in patients with myocardial infarction. Epidemiological studies have clearly shown elevated blood pressure to be a certain risk factor indeed, one of the most reliable examined (Lipstein 1965 1968 1973 b Semborg 1970 Fejfar 1972, Carlson & Böttiger 1971, Stamler 1973 Wilhelmsen *et al* 1973).

The Framingham prospective study (Dannel *et al* 1971 b) demonstrated that both systolic and diastolic blood pressures were strong predictors of the development of IHD and were independent of cigarette smoking and serum cholesterol levels. The risk was found to be proportional to the magnitude of all indices of arterial pressure, and no "safe" critical level could be identified. The level of casual blood pressure also appeared to be

a good predictor of IHD. Discriminant analysis disclosed that the systolic pressure's ability to predict future IHD improved with advancing age. This was not the case for diastolic pressure. Keys *et al* (1971) also found that systolic blood pressure was superior to diastolic blood pressure in the prediction of IHD death or myocardial infarction.

The risks presented by *mild hypertension* have been elucidated (Veterans Administration Cooperative Study Group 1970, Paul 1971 U.S. Public Health Service Hospitals Study Group 1972). In a pooled group of 6640 men, initially aged 30–59 from 6 different studies, Paul (1971) disclosed that a diastolic blood pressure of 85 to 104 mm Hg, as recorded at an initial examination, had unfavorable implications in terms of total death rate, deaths from IHD and non fatal myocardial infarction. The presence of elevated serum cholesterol and the smoking of cigarettes increased the risk of myocardial infarction and IHD death at essentially all blood pressure levels.

The effects of hypertension *treatment* have been studied e.g. by Mathiesen *et al* (1969) and the Veterans Administration Cooperative Study Group (1967 1970, 1972). In these studies the incidence of myocardial infarction and sudden death was essentially the same in the treated and control groups.

Blood pressure in relation to heredity

Using twins from a number of twin studies, on Verschuier (1958) found that 53% of the reported MZ pairs were concordant with respect to hypertension as opposed to 1.8% of the DZ pairs. In a study of 200 twin pairs, most of whom less than 5 years of age Hines *et al* (1957) found that intra-pair differences in both systolic and diastolic blood pressure were less for the MZ than

TABLE 16. Frequency of concordance and discordance with respect to previous diagnosis of hypertension in relation to alcohol consumption

		MZ			DZ			MZ+DZ		
		pos	neg	tot	pos	neg	tot	pos	neg	tot
HIGH ALC	pos	1	1	2	0	11*	11	1	12*	13
	neg	2	10	12	2	43	45	4	53	57
	tot	3	11	14	2	54	56	5	65	70

* $p < 0.05$

the DZ twins. In a study of smoking-discordant twin pairs Lundman (1966) found a significantly lower intra-pair variance in the MZ than in the DZ group with respect to both systolic and diastolic blood pressure. However the levels of significance in this study were considerably greater for the female group than the male group. In a study of 75 MZ and 84 DZ twin pairs aged 30-40 years, Takkinen (1964) found that systolic blood pressure proved to be subject to genetic influence when intra-pair variance in the two zygosity groups was compared. Diastolic blood pressure on the other hand, was judged to be mainly under environmental influence as no statistical significance was obtained. Deutscher *et al.* (1966) observed striking and increasing a resemblance between children and their parents with respect to systolic blood pressure after the age of 40.

Mathers *et al.* (1961) and Osborne *et al.* (1963) investigated 53 pairs of normotensive twins and measured their basal and casual blood pressure. Analysis of the data by comparing intra-pair variance in the MZ and DZ male groups failed to disclose the presence of any sizeable genetic factor. However a genetic factor was demonstrated for females. Downie *et al.* (1968) screening 81 young same-sexed twin pairs, concluded that no evidence of strong genetic component could be detected

by means of a single measurement of casual blood pressure. Nor was Liljefors (1970) able to demonstrate any genetic component for systolic or diastolic blood pressures when he analysed the apparently healthy twins in his study.

Alcohol and blood pressure

In apparently healthy subjects, moderate doses of alcohol are followed by a rather consistent but only transient and slight increase in heart rate, cardiac output and systolic and diastolic blood pressure (Grollman 1942 Holmberg & Mårtens 1955 Stein *et al.* 1963 Wallgren & Barry III 1970 Opaleva Stegantsheva *et al.* 1972) Marbach & Schwartz (1964) observed an increase in cardiac output without any change in blood pressure in humans after alcohol intake adjusting a decrease in vascular resistance. In experiments with dogs, Webb & Degerli (1965) reported decrease in peripheral resistance and venous pressure after the administration of large doses of alcohol.

The circulatory response to severe alcohol intoxication has been studied in animals and humans. Using rabbits Kakolewski & Humwich (1968) found that hypotensive reactions sometimes occurred, even leading to a fatal drop in blood pressure. It was suggested that inhibition of anti-diuretic hormone may contribute to the inability to

	MZ		No of pairs	DZ		No of pairs	MZ+DZ		N of pairs
	High alc. M(SD)	Low alc. M(SD)		High alc. M(SD)	Low alc. M(SD)		High alc. M(SD)	Low alc. M(SD)	
Total	158 (21.9)	159 (30.1)	14	158 (18.7)	148.9 (17.6)	56	158.1 (19.2)	151.0 (20.9)	70
Drinking > 1000 g/week	148 (21.1)	151 (5.3)	11	148.1 (16.3)	141 (17.3)	56	148.0 (17.2)	143.4 (21.3)	70
	158 (21.1)	151 (30.1)	5	157.2 (17.6)	147.1 (13.8)	25	157.4 (17.9)	147.8 (16.3)	30
	150 (18.9)	147 (30.9)	5	147.1 (13.6)	140.0 (15.3)	25	147.6 (14.3)	141.2 (18.2)	30

P = < 0.05 P = < 0.01

maintain blood pressure. In humans succumbing from alcohol intoxication, hypotension and circulatory failure are prominent features (Edmondson *et al* 1956 Wallgren & Barry III 1970).

Studies of *chronic alcoholics* with and without signs of cardiomyopathy have been reported by e.g. Asokan *et al* (1972) and Spodick *et al* (1972). The patients were studied long after all alcohol had been eliminated from the blood. In these investigations signs of impaired myocardial function were found in the form of low mean cardiac output, elevated mean left ventricular end diastolic pressure and a prolonged pre-ejection period. This was also true of the clinically normal alcoholic subjects. In the study by Spodick *et al* (1972) the clinically normal alcoholic patients had lower systolic and diastolic blood pressures than the control group. The subjects with alcohol induced cardiomyopathy displayed the lowest systolic and diastolic pressures. Alcoholic subjects with cirrhosis of the liver have been said to exhibit a lower incidence of hypertension than a control group (Hall *et al* 1953 Edmondson 1956, Sherlock 1968).

The symptoms associated with *unabused* include tremor, excessive perspiration, nervousness, weakness, gastric distress, anorexia, hyperreflexia, insomnia and elevated heart rate, body temperature and blood pressure (Cutshall 1965 Wallgren & Barry III 1970 Carlsson 1970). In a study of prolonged drinking and sudden withdrawal, Isbell *et al* (1955) found that symptoms and physical findings began to appear before the blood alcohol level had dropped to zero. Symptoms reached their peak 1 to 2 days after cessation of drinking. No evidence of residual impairment persisted three months after the termination of drinking.

In his study of high blood pressure in men aged 50 Tibblin (1967) found a significant and positive relationship between *alcohol problems* and blood pressure. Alcohol problems in this investigation were defined as at least one entry in the records of the local Temperance Board. * According to Pell & D'Alonzo (1973) the incidence of

* Sw. 'Nyktterhetsbussen'.

TABLE 18 Frequency of concordance and discordance with respect to casual and basal systolic blood pressures ≥ 140 mm Hg in relation to alcohol consumption

		LOW ALC									
		LZ			DZ			LZ+DZ			
		≥ 140	< 140	tot	≥ 140	< 140	tot	≥ 140	< 140	tot	
HIGH ALC	Casual systolic	≥ 140	8	3	11	33	17**	50	41	20**	61
		< 140	1	2	3	5	1	6	6	3	9
		tot	9	5	14	38	18	56	47	23	70
	Basal systolic	≥ 140	6	3	9	23	17*	40	29	20*	49
		< 140	3	2	5	7	9	16	10	11	21
		tot	9	5	14	30	26	56	39	31	70

* $p < 0.05$

** $p < 0.01$

hypertension over a five year period was 2.3 times higher for "working alcoholics" than for a matched control group. It was suggested that repeated elevations of the blood pressure after alcohol intake may cause a sustained high blood pressure (D'Alonzo and Pell 1968).

Moderate doses of alcohol in apparently healthy persons tend to produce a transient rise in blood pressure while severe alcohol intoxication leads to hypotension and circulatory failure. Subjects with alcohol problems have elevated blood pressures, while former alcoholics tend to have low blood pressures. The withdrawal period is generally characterized by elevated blood pressure. The effects of longstanding, moderate alcohol intake on blood pressure have apparently not, so far been elucidated.

MATERIAL AND METHODS

The subject group appears in chapter I. The twins had their casual and basal blood pressures measured as described in Methods (p. 19). After physical examination, questions regarding previous hypertension and antihypertensive treatment were

asked. Previous or present hypertension was accepted when previous or present treatment for high blood pressure was reported by the subject. Two subjects in the high alcohol group (HAG) and the same number in the low alcohol group (LAG) were being treated with antihypertensive agents at the time of the investigation. No twin pair was concordant with respect to present antihypertensive therapy. The twin pairs in which one of the subjects was under treatment were included in the results presented below.

RESULTS

Previous diagnosis of hypertension

An association between alcohol consumption and previous diagnosis of hypertension (Table 16) was found in the pooled zygosity group ($p < 0.01$). Thirteen of the subjects in the HAG versus 5 subjects in the LAG reported previous or present treatment for hypertension. One pair was concordant in this respect. The significant difference between the HAG and LAG was derived from the DZ group. The small number of subjects and the

	mm Hg		N of pairs	DZ		N of pairs	MZ+DZ		N of pairs
	High k	Low k		High k	Low k		High k	Low k	
	M(SD)			M(SD)			M(SD)		
Twin 1	Casual	Low k	11	High k	Low k	36	High k	Low k	70
	91.6(11)	93.0(13.7)		93.3(9.7)	89.3(9.3)		91.6(10.3)	90.2(10.3)	
Twin 2	Casual	Low k	11	High k	Low k	36	High k	Low k	70
	91.1(11)	91.6(13.3)		91.8(9.6)	86.3(9.0)		91.2(9.8)	87.5(10.1)	
DZ	Casual	Low k	3	High k	Low k	23	High k	Low k	30
	88.8(10.3)	91.1(12.6)		96.3(10.8)	87.8(9.3)		93.1(10.8)	88.1(9.7)	
MZ	Casual	Low k	3	High k	Low k	23	High k	Low k	30
	86.8(9.2)	89.1(11.1)		92.3(10.6)	81.8(9.7)		91.1(10.1)	85.3(10.1)	
	p = < 0.001			p = < 0.001			p = < 0.001		

low prevalence preclude the drawing of any conclusion for the MZ group.

Current level of blood pressure

Systolic blood pressure

A significant association was found between alcohol consumption and systolic blood pressure in the pooled zygosity group (Table 17). Thus, the twin with a high alcohol consumption (HAG) in a pair also had a higher average systolic blood pressure than the twin with a low alcohol consumption (LAG). This result was most pronounced in casual measurement ($p < 0.01$) but was even significant for the basal determination ($p < 0.05$). Mean values amounted to 158.1 and 151.0 mm Hg (casual), 148.0 and 143.4 mm Hg (basal) for the HAG and LAG respectively. Results applied to the DZ group ($p < 0.01$ and < 0.05). Mean values showed no obvious differences in the MZ group.













When the criterion of alcohol discordance was set at $> 10,000$ g/year (Table 17), systolic mean differences, both casual and basal, were more pronounced, implying a higher mean value for the HAG. This was true for both zygosity groups.

The pressure level limit was set at ≥ 140 mm Hg (Table 18) in evaluating the systolic intra-pair status. The significant connection between alcohol consumption and casual systolic blood pressure was also found in the pooled zygosity group ($p < 0.01$) when this limit was used. Casual systolic pressure ≥ 140 mm Hg was found in 61 HAG and 47 LAG subjects. There were 41 pairs in which both twins had a casual value of ≥ 140 mm Hg. That the association was significant was mainly due to results for the DZ group. Findings were consistent when basal values were analysed ($p < 0.05$).

Diastolic blood pressure

The most obvious finding was an association between alcohol consumption and both casual and basal diastolic blood pressure ($p < 0.001$) as seen in Table 19. This was true for the pooled zygosity

TABLE 20 Frequency of concordance and discordance with respect to casual and basal diastolic blood pressure ≥ 90 mm Hg in relation to alcohol consumption

		LOW ALC									
		MZ			DZ			tot			
		>90	<90	tot	>90	<90	tot	>90	<90	tot	
HIGH ALC	Casual diastolic	> 90	7 	9	32 	15**	47	39 	17*	56	
		< 90	 3	2	5	 3	6	9	 6	8	14
		tot	10	4	14	35	21	56	45	25	70
	Basal diastolic	> 90	5 	1	6	22 	16**	38	27 	17*	44
		< 90	 4	4	8	 3	15	18	 7	19	26
		tot	9	5	14	25	31	56	34	36	70

* = $p < 0.05$

** = $p < 0.01$

group in which mean values for diastolic blood pressure were 94.6 and 90.2 mm Hg (casual) and 91.2 and 87.5 mm Hg (basal) in the HAG and LAG respectively. Results were derived from the DZ group ($p < 0.001$). No such association was found in the MZ group.

Differences in the mean values of the diastolic blood pressures, both casual and basal, appeared to be more pronounced in the pooled zygoty group and among the DZ twins when the alcohol discordance criterion was greatest, $> 10,000$ g of absolute alcohol/year (Table 19).

In the evaluation of concordance and discordance with respect to casual diastolic blood pressure ≥ 90 mm Hg (Table 20) a significant association ($p < 0.05$) between alcohol consumption and

diastolic pressure was again found. Fifty-six and 45 of the HAG and the LAG respectively in the pooled zygoty group were above this limit. There were 39 pairs in which both twins in a pair had a casual value ≥ 90 mm Hg. That the association was significant was mainly due to the results from the DZ group ($p < 0.01$). Differences in basal diastolic pressure in the pooled zygoty group were mainly the same as those obtained for casual measurements ($p < 0.05$).

DISCUSSION

As has been shown, a high alcohol intake is accompanied by elevated blood pressure. Differences in the mean values increased with rising alcohol discordance. This finding could be explained

TABLE 1 Right upper arm circumference (centimeters) in relation to alcohol consumption

MZ (N=14 pairs)		DZ (N=36 pairs)		MZ+DZ (N=70 pairs)	
High alc.	Low alc.	High alc.	Low alc.	High alc.	Low alc.
M(SD)		M(SD)		M(SD)	
29.0(2.5)	29.8(1.9)	28.7(2.4)	28.7(2.7)	28.7(2.4)	28.9(2.6)

by the presence of alcohol in blood (discussed e.g. by Holmberg & Mårtens 1955, Wallgren & Barry III 1970). However, this was so in only one of the 140 subjects examined. Withdrawal also gives rise to elevated blood pressure (Isbell *et al.* 1955, Cutshall 1965). However, the subjects were ignorant of the purpose of the study and so it may be assumed that their drinking habits had not changed in the weeks preceding the examination. On the other hand, they were ordered to fast from the evening prior to the day of examination. Even if some subjects with a high alcohol intake had stopped drinking because of travelling, etc., more than 24 hours prior to examination, sustained elevated catecholamine levels might explain the elevated blood pressure as shown by Broholt *et al.* (1970).

The finding of higher blood pressure levels in the alcohol positive group agreed with the history of previous or present hypertension given. This association is thus confirmed by other, in principle, independent investigators and other periods of time.

How an increased upper arm circumference results in an elevated auscultatory blood pressure has been discussed by Tibblin (1967) and Rose & Blackburn (1968). This could not explain elevated systolic and diastolic blood pressure found in the DZ group, since mean values for the upper arm circumference were identical in the high and low alcohol groups (Table 21). However, a lower right upper arm circumference was found for the high alcohol consumers in the MZ group. This difference was not significant using a paired *t*-test

but was probably not random. When the MZ pairs were tested using the McNemar non-parametric test (Siegel 1956) a significantly larger number of the alcohol-positive twins had circumferences less than their co-twins. Apart from genetic considerations, this might explain blood pressure results in the MZ group.

It must be emphasized that the number of subjects in the monozygotic group, in which no association was found, was small. The selection of MZ twin pairs exhibiting discordance > 5000 g of absolute alcohol/year produced 10 pairs. The high alcohol consuming twins in 7 pairs displayed a higher casual diastolic blood pressure than their co-twins. The reverse was found in 3 pairs. This might mean that the association between alcohol consumption and blood pressure may persist even when the influence of genetic factors is minimal.

SUMMARY

The blood pressures in seventy alcohol-discordant twin pairs were examined. In the pooled zygosity group (MZ+DZ) a significantly greater proportion of the high consumers had a previous history of hypertension than the low alcohol consumers. A significant association was also found between alcohol consumption and the current blood pressures, both systolic and diastolic. The findings were independent of whether measurements were casual or basal. These results point to an association between moderate, longstanding alcohol consumption and elevated blood pressure when the influence of genetic and early environmental factors is assumed to be minimal.

Weight and skinfold thickness

A number of epidemiological investigations have shown a link between obesity and morbidity and mortality due to IHD. However, obesity appears to be a factor of limited importance. It is supposed that the factors associated with IHD e.g. over weight, may be partly explained by a high alcohol consumption. There is little information about the relationship between alcohol consumption and weight. The purpose of this chapter is to elucidate the relationship between longstanding alcohol consumption and weight (absolute and relative) and skinfold thickness when the influence of genetic and early environmental factors is minimal.

PREVIOUS STUDIES

Weight and IHD

Long before the current preoccupation with risk factors for IHD American life insurance companies were able to demonstrate increased mortality due to atherosclerotic disease in overweight subjects (Dublin 1930). Master *et al* (1953) found an increased incidence of overweight in men for all forms of IHD as compared to a control group. Several investigations have disclosed a relationship between body build, "mesomorphy" and IHD (e.g. Gertler & White 1954, Walker & Gregoratus 1967). However the heavy weight in these studies was not a manifestation of obesity but mesomorphic body build. The Framingham prospective study (Kannel *et al* 1967a) comprising more than 5,000 subjects aged 30–62 years at entry revealed an excess risk for angina pectoris and sudden death for obese men both with and without elevated blood pressure and serum cholesterol. Thus, these results indicate an independent contribution by obesity to the rate of development of some manifestations of IHD. According to Epstein (1965) there is little doubt

that obesity per se is a less potent risk factor than such a factor as serum cholesterol or the level of blood pressure. In his review Simonson (1970) concluded that overweight subjects "have an increased risk of angina pectoris and sudden death not due to myocardial infarction."

In the seven-year follow-up of Evans County (Heyden *et al* 1971) in which smokers were compared to non-smokers, the risk of developing IHD did not increase from the leanest to the most obese non-smokers. However a link between over weight and IHD was apparent in smokers. Keys *et al* (1972b) studying more than 10 000 middle-aged men with multivariate analysis (multiple logistic equation) was able to show that no measure of relative weight or obesity made any significant contribution to future IHD irrespective of age, blood pressure, serum cholesterol and smoking.

Weight and heredity

In a study of male twins aged 30–40 years, i.e. 75 MZ and 84 DZ twins, Takkunen (1964) found that skeletal dimensions were chiefly influenced by heredity. Both upper arm and subscapular skinfold thickness were poor measures of hereditary influence and were almost exclusively dependent on environmental factors. Weight was found to be dependent on both hereditary and environmental factors. Similar results were obtained by Lundman (1966) and Liljeferg (1970) for weight.

Weight and alcohol consumption

There is little information for humans concerning weight in relation to alcohol consumption. In a study of healthy and gainfully employed middle-aged men, it was found that the heaviest men consumed the largest quantities of alcohol (Toffler *et al* 1969) and weight gain was correlated to

heavy current beer and alcohol consumption. In a comparison of overweight in alcoholics and controls in an American company D Alonzo and Pell (1968) found that the proportion of drinkers and controls who were 20 % or more over their ideal weight was about the same. However a significantly larger percentage of drinkers were below ideal weight (< 0.9) that is 18.2 % as compared in 11.4 %. Eriksson (1969) found a lower body weight in heavy-drinking rat strains than in low consumption strains. The heavy-drinking strain also had a larger intake of solid food, suggesting a higher level of general metabolism for the heavy drinkers.

MATERIAL AND METHODS

The subject group appears in chapter I. The twins had their weight and skinfold thickness measured as described in Methods (p. 19). Their relative weight was determined according to the following equation:

$$\text{weight (kg)} =$$

$$0.9 (\text{height, cm} - 100)$$

Overweight was defined as a quotient ≥ 1.20 .

RESULTS

Absolute and relative weight

The main finding of this study was the lack of any link between absolute weight and alcohol consumption (Table 22). The difference in mean values in the highest discordance class (A) for absolute weight amounted to 73.4 and 70.0 kg in the HAG and LAG respectively.

A analysis of relative weight (Table 23) failed to disclose any significant disparities between the high and low alcohol consumers. In the highest discordance class (A) however an increased number of alcohol positive twins had a relative weight quotient ≥ 1.20 .

Skinfold thickness

A significantly thicker layer of subcutaneous fat was found in the twins reared together (Table 24) in the highest discordance class (A) for the high alcohol consumers ($p = 0.05$) in the pooled zygosity

	No of pairs	MZ+DZ	
		High alc	Low alc
		M(SD)	
	25	73.4(12.7)	70.0(11.3)
	11	73.4(11.5)	71.1(10.8)
	13	72.8(11.6)	76.6(13.5)
	36	3.9(11.9)	72.9(11.9)

	No of pairs	DZ	
		High alc	Low alc
		M(SD)	
	25	73.8(11.1)	69.9(10.9)
	11	71.3(12.5)	72.6(10.4)
	13	71.1(12.5)	77.4(14.4)
	36	71.0(11.7)	72.5(11.8)

Cl	Discordance in the %/ year (arrest)	No of pairs	MZ	
			High alc	Low alc
			M(SD)	
A	> 10,000	3	71.4(20.6)	70.4(14.5)
B	5,000-10,000	3	79.0(6.7)	79.1(12.0)
C	< 5,000	1	68.5(8.6)	71.0(11.1)
Total		14	73.3(15.5)	71.6(11.6)

TABLE 25 Frequency of concordance and discordance in respect to relative weight ≥ 1.20 in relation to alcohol consumption

		LOW ALC									
		MZ			DZ			MZ+DZ			
		≥ 1	$20 < 1$	20 tot	≥ 1	$20 < 1$	20 tot	≥ 1	$20 < 1$	20 tot	
Total	≥ 1	20	4	1	5	4	10	14	8	13	19
	< 1	20	2	7	9	8	34	42	10	41	51
	tot		6	8	14	12	44	56	18	52	70
HIGH ALC											
Discordance $> 10,000$ g/year	≥ 1	20	2	0	2	1	0	1	3	0	10
	< 1	20	1	2	3	2	15	17	3	17	20
	tot		3	2	5	3	22	25	6	24	30

group. This finding was not noted in the two lower discordance classes (B and C).

No significant disparities were found in the subscapular area (Table 25) in high and low consumers of alcohol.

DISCUSSION

The present study found no significant positive association between alcohol consumption and increased absolute or relative weight. Such an hypothesis has received but little support in previous reports, due, perhaps to a failure to report negative findings.

Differing behaviour regarding the type and fre-

quency of alcohol consumption may have different effects on weight. German beer drinkers, for instance, are known to be obese. In a study of 8 young (22–26 years) healthy volunteers, prolonged beer administration, corresponding to 63 grams of absolute alcohol/day increased body weight in all cases but one during an experimental period of 5 weeks (Belfrage *et al* 1973). On the other hand, subjects with a heavy consumption of hard liquor and a poor diet may be lean. When heavy alcohol consumption is accompanied by somatic complications, such as gastric ulcer, pancreatitis and malabsorption (Carlsson 1970, Wallgren & Barry III 1970) affected subjects often have a lower-than-a-crage weight.

TABLE 3 Skinfold thickness in triceps area (mm) in relation to alcohol consumption

Class	Discordance in abs alc/ year (grams)	MZ			DZ			MZ+DZ		
		High alc.	Low alc.	No. of pairs	High alc.	Low alc.	No. of pairs	High alc.	Low alc.	No. of pairs
		M(SD)			M(SD)			M(SD)		
A	$> 10,000$	8.3(3.3)	8.6(3.9)	5	9.4(4.8)	7.7(3.0)	25	9.2(4.3)	7.8(3.1)	30
B	5,000–10,000	10.6(3.0)	12.9(11.3)	5	8.5(2.8)	8.5(2.7)	18	8.9(2.9)	9.3(5.7)	23
C	$< 5,000$	8.4(2.2)	10.6(3.2)	4	9.1(2.9)	9.8(3.5)	15	8.9(2.7)	10.0(2.6)	17
Total		9.0(2.9)	10.7(7.1)	14	9.0(3.8)	8.4(2.9)	36	9.0(3.6)	8.9(4.1)	70

$= p < 0.05$

TABLE 25 Skinfold thickness in subscapular area (mm) in relation to alcohol consumption

Class	Discordance in abs. alc/ years (grams)	MZ			DZ			MZ+DZ		
		High alc.	Low alc.	N of pairs	High alc.	Low alc.	No. of pairs	High alc.	Low alc.	No. of pairs
		M(SD)			M(SD)			M(SD)		
A	> 10,000	16.0(5.4)	15.2(2.1)	5	17.4(6.0)	15.3(7.1)	25	17.2(5.8)	15.3(6.5)	30
B	5,000-10,000	21.6(9.0)	21.9(9.5)	5	16.5(6.5)	15.7(4.4)	18	18.0(7.6)	17.0(6.2)	25
C	< 5,000	13.1(4.8)	17.8(5.5)	4	17.1(5.6)	19.9(6.6)	15	16.2(5.6)	19.4(6.0)	17
	Total	17.9(7.8)	18.5(6.5)	14	17.0(6.0)	16.5(6.4)	56	17.2(6.5)	16.9(6.4)	70

Despite the absence of any link between alcohol consumption and weight in the present study a positive and significant relationship was noted between subcutaneous fat thickness in the triceps area and alcohol intake in the highest discordance class. A similar trend with respect to the subscapular area was also found. The fact that skinfold thickness only was of significance in accordance with the assumption that this parameter has a more exclusively environmental origin (Takkinen 1964, Liljeboom 1970). However results were too heterogeneous to support any obvious relationship between obesity and unspecified alcohol consumption, at least as long as the influence of genetic and early environmental factors was assumed to be minimal.

SUMMARY

Seventy alcohol-discordant twin pairs were examined with respect to absolute and relative weight and skinfold thickness. No significant link was found between alcohol consumption and any measure of weight. The alcohol-positive twins in the highest discordance class of the pooled zygosity group (MZ+DZ) had a significantly thicker layer of subcutaneous fat in the triceps area than their low consumption co-twins. No differences were found for the subscapular area. It is concluded that no obvious relationship between unspecified alcohol consumption and obesity can be demonstrated when the influence of genetic and early environmental factors is expected to be minimized.

Serum lipids

An association between various disorders of lipid metabolism and IHD has been established. It is assumed that variables associated with IHD such as serum lipid levels, may be explained to some extent by alcohol habits. In clinical experiments, acute and prolonged alcohol administration to healthy subjects has been reported to increase the level of serum lipids. Intense lipidaemia has also been recognized in alcoholics. The purpose of this chapter is to consider the possible action of prolonged, moderate alcohol consumption on the amounts of lipoproteins and lipids in serum when the influence of constitutional factors is assumed to be minimal.

PREVIOUS STUDIES

Cholesterol and IHD

In investigations using laboratory animals the severity of atherosclerotic lesions has been found to increase with increasing cholesterol levels (Malmros & Wigand 1959). Clinical studies have shown a significantly increased number of subjects with elevated serum cholesterol in groups suffering from IHD as compared to control groups (Bisbeck *et al* 1957 b, Arrow *et al* 1973, Goldstein *et al* 1973). This applies especially to younger age groups.

The close connection between elevated cholesterol levels and IHD is also apparent from a number of reviews and prospective epidemiological studies (Epstein 1965, Sanborg 1970, Fejfar 1972, Stamler 1973). A linear relationship was found between cholesterol level and IHD when incidence data from studies in 7 countries were compared (Key 1970). The Framingham prospective study (Kannel *et al* 1971 a) noted a stronger positive association between IHD and serum cholesterol in younger persons than in older persons. Further

more, the risk of each clinical manifestation of IHD proved proportional to the antecedent cholesterol level. The Stockholm prospective study (Carlson & Böttiger 1972) found that the IHD rate was directly proportional to fasting concentrations of serum cholesterol in subjects under 60 years of age, but not in an older age-group. Using a multiple logistic model, Wilhelmsen *et al* (1973) found that the presence of high serum cholesterol levels as compared to 8 other variables was the most reliable, independent, discriminating factor in the development of non fatal myocardial infarction and acute IHD death.

Triglycerides and IHD

The literature on serum triglycerides and IHD is not very extensive but suggests a link between a high level for serum triglycerides and IHD. Albrink & Man (1959) reported that serum triglyceride levels were more often elevated in patients with IHD than in healthy subjects. Carlson (1960) found significantly elevated levels of serum triglycerides in men under 50 years with infarction. In a study of lipid levels in 500 consecutively 3-month survivors of myocardial infarction, Goldstein *et al* (1973) were able to demonstrate that 31 % had hyperlipidaemia. Lipid abnormalities among the males were most commonly found in patients under 40. Furthermore, these authors showed that elevated serum triglycerides, with or without concomitant elevation of serum cholesterol, was three times more common in survivors than a high cholesterol level alone. In the Stockholm prospective study of slightly more than 3 000 men (Carlson & Böttiger 1972) the rate of IHD was directly proportional to increasing fasting concentrations of serum triglycerides. Both serum triglycerides and serum cholesterol were found to be independent risk factors in IHD but

significant differences for both lipids could only be shown for subjects under 60 years of age.

Serum lipoproteins and IHD

Several investigators have emphasized that it is inadequate to classify basic serum lipoprotein abnormalities simply by reference to elevated serum cholesterol or serum triglycerides. Thus, Fredrickson & Lees (1965) suggested a hyperlipoproteinemia classification system, which was modified in 1970 in accordance with Beumont *et al*. This system provides a method of systematically classifying the lipoproteins. The hyperlipoproteinemias of major interest in connection with IHD are Types II A, II B, III and IV (Gustafson 1973). Type II A denotes an increase in serum cholesterol. This is seen as a beta-lipoprotein pattern in lipid electrophoresis. In types II B and III there is an increase both in serum cholesterol and a rise in serum triglycerides presenting as a mixture of beta and pre-beta patterns in electrophoresis. Type IV denotes an increased triglyceride level, a moderate elevation of serum cholesterol values and a pre-beta pattern.

Blood lipids in relation to heredity

Several investigators have disclosed a varying degree of genetic influence on serum cholesterol and phospholipids in man but there is little data on serum triglycerides and lipoproteins.

The importance of genetic factors on serum cholesterol levels has been firmly established (Wilkinson *et al* 1948) in respect to primary hypercholesterolaemia (II A). In a study of 34 twin pairs, Meyer (1962) found a significantly smaller intra pair variance for serum cholesterol in MZ than DZ pairs living together thus indicating a genetic influence. On the other hand, twins living apart displayed variances exceeding those displayed by twins living together thereby demonstrating the importance of environmental factors as well. Similar results were reported by Osborn *et al* (1959) McDoonough *et al* (1962) Jensen *et al* (1965) and Rifkind *et al* (1968). Lundman (1966) found an intra pair variance for serum cholesterol which was lower in MZ than in DZ

twins but not significantly so. Liljefors (1970) revealed an apparent genetic influence on cholesterol levels in terms of intra pair variance comparison. Heiberg (1973) also found an obvious genetic influence on cholesterol levels, and the use of H statistics suggested that the variation for men was genetically determined to 84 %.

Lundman (1966) found lower intra-pair variances for MZ than for DZ twins with respect to serum triglycerides. However significant differences were only found for females. The subject group used by Liljefors (1970) consisted exclusively of males, and he was unable to show any definite genetic effect on the triglyceride level, which varied to the same extent within pairs, irrespective of zygosity. Nor could Heiberg (1973) find any formal evidence for genetic determination of serum triglyceride levels.

Alcohol and blood lipids

Prolonged, moderate administration of alcohol to 8 young volunteers only produced minor non-significant elevations of serum cholesterol levels (Berg & Johansson 1973). Adelson & Keys (1963) found that a moderate-to-high habitual intake of alcohol by 123 business and professional men was associated with elevated serum cholesterol values. Serum cholesterol in 7 chronic alcoholics was found to increase during prolonged administration of alcohol (Lieber *et al* 1963). Experimental studies using animals and reviewed by e.g. Hirst *et al* (1965) and Wallgren & Barry III (1970) have generally disclosed elevation of serum cholesterol during prolonged alcohol administration. Lefevre *et al* (1972) found an increase in serum cholesterol during alcohol feeding of rats and concluded that this was linked to both enhanced cholestero-genesis and decreased excretion of bile acids.

A large increase in serum triglycerides is often seen in subjects with alcohol abuse (Lieber *et al* 1963 Losowsky *et al* 1963 Clark *et al* 1972, Sartory *et al* 1972). Even a large single dose (Nestel *et al* 1965 Broholt *et al* 1970) or prolonged moderate alcohol intake (Berg & Johansson 1973) in apparently healthy subjects has been found to be

TABLE 26. Serum cholesterol in relation to alcohol consumption

Class	Discordance in abs. alc/ year (grams)	MZ			DZ			MZ+DZ		
		High alc.	Low alc.	No. of pairs	High alc.	Low alc.	No. of pairs	High alc.	Low alc.	No. of pairs
		M(SD)	M(SD)		M(SD)	M(SD)		M(SD)	M(SD)	
A	> 10,000	215(38)	229(75)	5	254(45)	223(43)	25	231(44)	225(48)	38
B	5,000-10,000	241(25)	262(68)	5	245(47)	238(55)	18	244(45)	245(57)	23
C	< 5,000	229(47)	230(33)	4	253(48)	238(55)	13	248(47)	236(43)	17
Total		228(36)	241(61)	14	242(46)	232(48)	56	239(44)	235(50)	70

accompanied by an increase in serum triglycerides. Mendelson & Mello (1973) found dramatic increases in serum triglycerides in alcoholic subjects with evidence of a type IV hyperlipoproteinemia as compared to normal controls following alcohol ingestion. This result and the findings of Losowsky *et al* (1963) suggest that the triglyceride response to alcohol intake is more pronounced in the presence of a preexisting lipoprotein derangement.

Lipoprotein patterns in alcoholics have been investigated by Johansson & Laurell (1969) who found an increase in alpha-lipoproteins. This finding was in accordance with Berg & Johansson (1973) who also found a slight but significant increase in alpha-lipoprotein levels in apparently healthy volunteers during prolonged moderate alcohol consumption. In all cases the increase was within normal limits. These studies showed no abnormalities in the other serum lipoproteins.

MATERIAL AND METHODS

The subject group is described in chapter I.

The tw pairs were examined with respect to serum cholesterol and serum triglycerides after an overnight fast. Lipoprotein patterns were determined for all twin pairs except the first 9. The methods applied are described on page 21.

RESULTS

Serum cholesterol

The mean values for serum cholesterol in the pooled zygosity group (Table 26) were not significantly different in the high and low consumers

of alcohol. These were 239 and 235 mg/100 ml in the HAG and LAG respectively. A higher mean cholesterol level was consistently found in the HAG in the three discordance classes (A-C). Sixty-one and 51 subjects in the HAG and LAG respectively displayed serum cholesterol values > 200 mg/100 ml (Table 27). There was concordance in 47 pairs on the basis of this criterion. In the 18 twin pairs in which one of the members had a serum cholesterol value > 200 mg/100 ml, the value in 14 pairs applied to the high consumer group ($p < 0.05$). An identical tendency was found when the limit for serum cholesterol was set at 250 mg/100 ml (Table 27).

Serum triglycerides

The high and low consumers in the pooled zygosity group showed the same mean value of triglycerides, i.e. 147 mg/100 ml (Table 28). In the highest discordance class (A) however an association between alcohol consumption and the level of triglycerides was suggested as mean values amounted to 140 and 122 mg/100 ml in the HAG and LAG respectively. However this was not significant. Furthermore, no such association could be found in the other discordance classes (B and C). Twenty-seven and 24 subjects in the HAG and LAG respectively exceeded the 150 mg/100 ml limit when intra pair comparison was made of the level of triglycerides (Table 29). The result was consistent even when the limit was set at > 200 mg/100 ml. Thus, these findings suggest that there is no association between habitual alcohol consumption and the fasting triglyceride level.

TABLE 7 Frequency of concordance and discordance with respect to serum cholesterol > 200 mg/100 ml and > 250 mg/100 ml in relation to alcohol consumption

	LOW ALC								
	MZ			DZ			MZ+DZ		
	> 200	≤ 200	tot	> 200	≤ 200	tot	> 200	≤ 200	tot
> 200	9	3	12	38	11	49	47	14*	61
≤ 200	6	8	14	4	3	7	4	5	9
tot	9	5	14	42	14	56	51	19	70

HIGH ALC									
	MZ			DZ			MZ+DZ		
	> 250	≤ 250	tot	> 250	≤ 250	tot	> 250	≤ 250	tot
> 250	2	2	4	9	13*	20	11	13	24
≤ 250	2	8	10	3	33	36	5	41	46
tot	4	10	14	12	44	56	16	54	70

* = $p < 0.05$

Serum lipoproteins

Negligible differences were found in lipoprotein levels. If a ≥ 60 limit for a pathological beta lipoprotein level was used, 23 subjects in the HAG and 4 subjects in the LAG were found to exceed this level (Table 30). The same was true for the pre-beta lipoproteins as 11 and 10 subjects in the HAG and LAG respectively exceeded the 5% value used as a limit for a pathological pre beta lipoprotein level (Table 31). The results

were heterogeneous with respect to alpha-lipoprotein and also failed to show any differences between high and low alcohol consumers.

DISCUSSION

A significant association of cholesterol levels exceeding 200 mg/100 ml in the pooled zygosity group and alcohol consumption was demonstrated. This relationship was not especially reliable as no significance was disclosed when intra pair differ

TABLE 28 Serum triglycerides in relation to alcohol consumption

Class	Discordance in absolute value (grams)	MZ			DZ			MZ+DZ		
		High alc.	Low alc.	N of pairs	High alc.	Low alc.	N of pairs	High alc.	Low alc.	N of pairs
		M(SD)			M(SD)			M(SD)		
A	> 1000	136(4)	140(5)	5	13(93)	118(41)	7	110(89)	122(10)	30
B	500-1000	119(14)	114(9)	5	161(100)	193(84)	18	153(90)	164(83)	3
C	< 500	132(4)	131(5)	4	133(61)	144(5)	13	149(56)	151(68)	17
Total		135(49)	137(57)	14	150(72)	149(4)	36	147(82)	147(68)	70

TABLE 29 Frequency of concordance and discordance with respect to triglycerid level >150 mg/100 ml and >200 mg/100 ml in relation to alcohol consumption.

	LOW ALC								
	MZ			DZ			MZ+DZ		
	>150	≤ 150	tot	>150	≤ 150	tot	>150	≤ 150	tot
>150	3	1	4	11	12	23	14	13	27
≤ 150	1	9	10	9	24	33	10	33	43
tot	4	10	14	20	36	56	24	46	70

HIGH ALC									
	>200			>200			>200		
	>200	≤ 200	tot	>200	≤ 200	tot	>200	≤ 200	tot
>200	0	2	2	4	7	11	4	9	13
≤ 200	1	11	12	9	36	45	10	47	57
tot	1	13	14	13	43	56	14	56	70

ences were assessed using the paired "t" test. However in view of the fact that the high alcohol consumers were to a significant extent heavier smokers (chapter IV) a finding in accordance with earlier studies (e.g. Friberg *et al.* 1973) the results here shown are not without interest. Lundman (1966) was able to demonstrate constant reduction of serum cholesterol in smoking subjects in his earlier twin study of smoking discordant twins. Furthermore, the link between alcohol con-

sumption and serum cholesterol might have been more pronounced if casual levels had been investigated. In earlier studies, during and immediately after a very large alcohol intake, elevated serum cholesterol levels have been demonstrated (Lieber *et al.* 1963 Losowsky *et al.* 1963). A normalization of serum cholesterol levels 1—2 days after the cessation of alcohol intake was generally found in these reports. In the present subject group, it is presumed that an overwhelming ma-

TABLE 30 Frequency of concordance and discordance with respect to beta lipoprotein level ≥ 60 per cent in relation to alcohol consumption

LOW ALC										
	MZ			DZ			MZ+DZ			
	>60	<60	tot	>60	<60	tot	>60	<60	tot	
HIGH ALC	>60	1	4	5	9	9	18	10	13	23
	<60	3	5	8	11	19	30	14	24	38
	tot	4	9	13	20	28	48	24	37	61

TABLE 31 Frequency of concordance and discordance between pre-beta lipoprotein level ≥ 5 per cent in relation to alcohol intake

		LOW ALC					
		KZ			DZ		
		≥ 25	< 25	tot	≥ 25	< 25	tot
HIGH ALC	≥ 5	1	2	3	4	4	8
	< 5	1	9	10	4	36	40
	tot	2	11	13	8	40	48
		KZ+DZ					
		≥ 25	< 25	tot			
HIGH ALC	≥ 5	5	6	11			
	< 5	5	45	50			
	tot	10	51	61			

portion of the high alcohol consumers were abstinent for 12–24 h prior to the drawing of blood samples. Despite the fact that many subjects in the alcohol positive group might be exposed to repeated elevations of serum cholesterol, a high level was not apparently maintained after abstinence.

According to the present study moderate alcohol consumption does not appear to significantly alter the level of triglycerides when measurements are made during a period of abstinence. However the immediate effect of alcohol on serum triglycerides, as reported in alcoholic and healthy subjects (Lieber *et al* 1963 Losowsky *et al* 1963 Broholt *et al* 1966 Berg & Johansson 1973) was also demonstrated in the present study. The one subject who had measurable blood alcohol (1.4 ‰) and who belonged to the alcohol-positive group had the highest triglyceride value in all determinations, i.e. 500 mg/100 ml. This subject, who also belonged to the highest DZ twin discordance class (A) was to some extent responsible for the tendency towards an elevated triglyceride level for the high alcohol consumers. In two twin pairs in the subject group whose fasting serum lipids were followed for five days, marked fluctuations especially in serum triglycerides were noted for the alcohol positive twins (Fig. 2).

No obvious population differences were found between high and low alcohol consumers. These findings may be due to genetic influences, as claimed by Heiberg (1973) and others or to the fact that the high consumers were abstinent the

day or days before investigation. It seems reasonable to assume that a fasting lipid value does not give a true picture in many cases of the lipid levels of a subject with a high habitual alcohol consumption. Hypertriglyceridaemia two or three times a week with a concomitant elevation in serum cho-

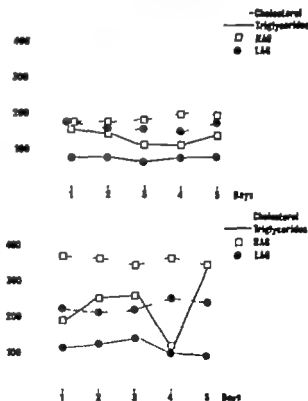


Fig. 2. Serum cholesterol and serum triglyceride level in mg/100 ml each twin pair during 5 days. Top pair (LAL) and bottom pair (HAL) are 33 and 34 years old.

lesterol may be overlooked if the subject remains abstinent one or two days before blood samples are drawn. A quick normalization of the triglyceride level has been reported in alcoholics while in hospital (Chait *et al* 1972). Casual lipid values have been analysed in epidemiological studies of serum lipids and IHD such as the Framingham prospective study (Kannel *et al* 1971 a). The results from casual measurements could imply that part of the association between serum lipids and IHD is explained by alcohol consumption.

SUMMARY

Seventy alcohol-discordant twin pairs were in-

vestigated with respect to serum cholesterol, triglyceride level and lipoprotein patterns. The data suggest a link between longstanding, moderate alcohol consumption and elevated serum cholesterol. Significantly higher numbers of the high alcohol consumers in the pooled zygosity group (MZ+DZ) displayed serum cholesterol values exceeding 200 mg/100 ml. No obvious disparities were revealed in either serum triglycerides or lipoprotein patterns. It is suggested that longstanding, moderate alcohol consumption is not apparently linked to elevated fasting serum lipids when the influence of genetic and early environmental factors is minimal.

Blood glucose and intravenous glucose tolerance

The association between IHD and various disorders of glucose metabolism is well known. It is assumed that variables associated with IHD such as the blood glucose level and intravenous glucose tolerance (IVG) may be due in part to a high alcohol intake. Alcohol administration in humans has been reported to cause a transient elevation in blood glucose levels, and an abnormal glucose metabolism has been shown to characterize "alcoholic subjects." The purpose of this chapter is to analyse the possible effects of a longstanding alcohol consumption on fasting blood glucose levels and intravenous glucose tolerance when the influence of hereditary and early environmental factors is minimal.

PREVIOUS STUDIES

Overt diabetes and IHD

An overrepresentation of diabetes mellitus in subjects with myocardial infarction has been shown by e.g. Ekelström (1951) Londen (1952) Siemers *et al* (1961) and Wahlberg (1962). Entmacher *et al* (1964) was able to show that the incidence of coronary deaths in diabetics trebled from 1922 to 1961. Furthermore the long-term prognosis for diabetic survivors of myocardial infarction was less favourable than for non-diabetics, according to Björk *et al* (1957 a) Londen (1962) Siemers (1963) Partanen & Bradley (1963) Wahlberg (1966) and Paasilin (1970). In a recent study however Helmers (1973) was unable to demonstrate that the prognosis following myocardial infarction was any worse for diabetics.

Impaired glucose tolerance and IHD

The relationship between milder disorders of glucose metabolism and IHD has been the subject of great interest in the last ten years (Epstein 1963 1969 1973 a, b). A number of studies have

disclosed a high prevalence of decreased intravenous and oral glucose tolerance in patients with manifest IHD (Wahlberg 1966 Paasilin 1970). Most investigations of this kind have been based on survivors of myocardial infarction or patients with otherwise clearcut clinical evidence of IHD (for references, see Pyörälä 1973).

Additional information about the relationship between impaired glucose tolerance and IHD has been obtained from population studies. The results of oral glucose tolerance tests were analyzed in these studies in relation to clinical and ECG manifestations of IHD. Studies of this kind have been performed by e.g. Keen *et al* (1965) Ostrand *et al* (1965) Welborn *et al* (1969) and Pyörälä (1973). With the exception of the latter these studies, after excluding patients with overt diabetes, disclosed a correlation between hyperglycaemia and IHD. In Pyörälä's prospective study (1973) of 1 269 Finnish policemen, no definite relationship was found between hyperglycaemia and the prevalence of angina pectoris or possible infarction according to Rose's questionnaire (1966). However ECG abnormalities displayed an increased prevalence with rising blood glucose levels in the 30—49 year age group.

Hyperglycaemia in relation to heredity

Diabetes mellitus is generally accepted as being, at least to some extent, an inherited disease (Cerasi 1967 Marble *et al* 1971) as shown by an increased concordance for the disease in identical twins and an increased prevalence in the offspring of diabetics.

Hyperglycaemia and glycosuria are late signs of the disorder and the overt phase of the disease is preceded by latent or silent stages revealed by glucose tolerance tests (Cerasi 1967). Then Berg (1938) investigated 133 initially diabetes-free

TABLE 32. Fasting blood glucose in mg/100 ml in relation to alcohol consumption

Class	Discordance in abs. alc/ year (grams)	MZ			DZ			MZ+DZ		
		High alc.	Low alc.	No. of pairs	High alc.	Low alc.	No. of pairs	High alc.	Low alc.	No. of pairs
		M(SD)			M(SD)			M(SD)		
A	> 10,000	95(13)	88(18)	5	82(16)	79(13)	25	84(16)	81(14)	50
B	5,000—10,000	84(14)	83(11)	5	81(21)	80(7)	18	84(19)	81(8)	23
C	< 5,000	71(8)	73(10)	4	89(20)	78(9)	13	85(19)	77(9)	17
	Total	84(13)	82(14)	14	84(19)	79(10)	56	84(18)	80(11)	70

 $\equiv p < 0.05$

dant twin pairs for evidence of latent diabetes in the clinically normal co-twin. She found that diabetes or latent diabetes was present in both members in 65 % of the MZ and 20 % of the DZ. Furthermore, both twins had diabetes in all MZ pairs more than 43 years of age. Gottlieb & Root (1968) studied 104 twin pairs from the Joslin Clinic and found a high concordance for overt diabetes in MZ twins in which the proband was 40 or more at the time of diagnosis. Similar results have been reported by Harvald & Hauge (1963) who found concordance for overt diabetes in 60 % of MZ versus 13 % of DZ same-sexed twins. Cerasi & Luft (1967) studied MZ twin pairs consisting of one diabetic and one member with a normal glucose tolerance. A striking similarity was found between their low insulin responses after glucose infusion, pointing to a genetic influence. However, the occurrence of overt diabetes, elevated fasting blood glucose or impaired

glucose tolerance are also governed by external influences.

Alcohol and glucose metabolism

The relation between glucose metabolism and alcohol was reviewed by Wallgren & Barry III (1970). Blood glucose levels rise after the ingestion of alcohol, according to the amount of alcohol ingested and on the magnitude of glycogen reserves. The release of glucose from hepatic glycogen reserves into the blood is governed at least in part by the adrenal medullosympathetic nervous system (Perman 1962, Brobuck *et al.* 1970) since the response is inhibited by cutting or blocking of the splanchnic nerves (Perman 1962). Peripheral utilization of glucose also diminishes after alcohol ingestion (Hed & Nygren 1968).

Wallgren & Barry III (1970) were unable to find any consistent abnormalities in the glucose metabolism of alcoholics. On the other hand,

TABLE 53 Frequency of concordance and discordance with respect to fasting blood glucose > 100 mg/100 ml relative to alcohol on 12/10/70

		LOW ALC								
		M2			D2			M2+D2		
		>100	≤100	tot	>100	≤100	tot	>100	≤100	tot
HIGH ALC	>100	0	3	3	0	6**	6	0	11**	11
	≤100	1	10	11	0	48	48	1	58	59
	tot	1	13	14	0	56	56	1	69	70

 $\alpha = 0.01$

Cl	Discontinue mg/kg/ per min	MZ			No. of pairs	DZ			No. of pairs	MZ+DZ			No. of pairs
		High	Low	M(SD)		High	Low	M(SD)		High	Low	M(SD)	
A	100	110(0.31)	111(0.32)	1	5	113(0.36)	116(0.34)	1	5	113(0.34)	121(0.34)	1	50
	R	185-0.70	143-0.77			2.9-0.70	1.9-0.68			2.29-0.70	1.98-0.68		
B	200	111(0.30)	110(0.33)	1	5	131(0.60)	132(0.63)	1	18	137(0.55)	150(0.57)	1	23
	R	149-0.5	181-1.02			2.92-0.20	3.73-0.84			1.92-0.20	3.73-0.84		
C	< 1000	230(2.02)	168(0.76)	1	4	113(0.60)	133(0.37)	1	13	140(1.07)	141(0.48)	1	17
	R	532-1.04	2.60-0.99			1.74-0.32	2.39-0.99			5.32-0.32	2.60-0.99		
	T	154(1.17)	138(0.51)	1	14	139(0.46)	136(0.37)	1	56	121(0.67)	136(0.17)	1	70
	R	532-0.70	2.60-0.77			2.92-0.20	3.73-0.69			3.32-0.70	3.73-0.68		

P < 0.05

according to Hed (1958 a, b) and Lundquist (1963) alcoholics have a higher than normal prevalence of abnormal glucose tolerance especially in conjunction with liver derangement and increasing age. Hed (1958 a) found diabetes mellitus or fasting blood sugar > 130 mg/100 ml in 26.5% of 67 males with portal cirrhosis of the liver mainly induced by alcohol. Oral glucose tolerance tests in 24 chronic alcoholics 7-10 days after admission to hospital (Hed 1958 b) revealed

diabetogenic disturbances in 12. Ten of these subjects had cirrhosis and 2 had pronounced fatty degeneration of the liver. Lundquist (1963) studied intravenous glucose tolerance (IVG) in 102 alcoholics 1-3 weeks after a drinking bout and in 27 alcoholics in a non-acute phase (hospitalized for not less than one month). Forty-one per cent of the 102 alcoholics had a k value less than 0.90, the incidence increasing with advancing age. None of the 27 patients in a post alcoholic phase had a pathological k value. Lundquist concluded that both the abnormal glucose tolerance and the pathological liver function tests could be normalized during hospital stay. However the reversibility of impaired glucose tolerance in alcoholics during a hospital stay as also observed by Phillips & Safrit (1971) suggests that severe irreversible liver damage as cirrhosis is not a prerequisite for disturbed carbohydrate metabolism. A number of authors have demonstrated (Dornhorst & Ouyang 1971, Phillips 1971, Shanley *et al* 1972, Waparc & Jones 1972, Dundee 1972) that the administration of alcohol also impairs glucose tolerance in normal subjects.

MATERIAL AND METHODS

The subject group appears in chapter 1. The methods applied are given in chapter II.

RESULTS

Fasting blood glucose

A significant association was found between fasting blood glucose in the pooled zygosity group and alcohol consumption (Table 3). Thus the twin in a pair with a high alcohol consumption (HAG) also had a higher average fasting blood

TABLE 35 Distribution of *k*-value (*k* ≤ 1: borderline and normal) in relation to alcohol consumption

<i>k</i> -value	MZ (14 pairs)				DZ (36 pairs)				MZ+DZ (70 pairs)			
	High alc.		Low alc.		High alc.		Low alc.		High alc.		Low alc.	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Diabetic	4	29		14	11	20	7	13	15	22	9	13
Borderline	3	21	2	14	19	34	8	14	22	31	10	14
Normal	7	50	10	77	26	46	41	73	53	47	51	5

glucose value than the twin with a low alcohol consumption (LAG). Mean values amounted to 84 and 80 mg/100 ml in the HAG and LAG respectively ($p < 0.05$). When the pooled zygosity group was separated into different discordance classes, a consistently higher mean value was found in the HAG than in the LAG. This was true also for the DZ group, in which the differences were most pronounced in class C. The difference was not significant for this discordance class nor for the total DZ group when it was tested using a paired *t*-test, but was probably not random, as the non-parametric McNemar's test (Siegel 1956) revealed significant differences ($p < 0.05$). The greatest difference for the MZ group was found in the highest discordance class (A) in which mean values amounted to 95 and 88 mg/100 ml in the HAG and LAG respectively.

When fasting blood glucose was analysed with respect to a limit set at > 100 mg/100 ml (Table

35) 11 HAG twins in the pooled zygosity group exceeded this limit as opposed to one LAG twin ($p < 0.01$). No twin pair was concordant as to blood glucose > 100 mg/100 ml in either zygosity group.

Intravenous glucose tolerance

A significantly lower *k* value was found for intravenous glucose tolerance in the HAG than in the LAG for the pooled zygosity group (Table 34). Mean values were 1.24 and 1.36 for the HAG and LAG respectively ($p < 0.05$). Lower *k* values were consistently found for the HAG than the LAG in all discordance classes in the DZ group. Results for the MZ group were contrary to expectations, due to the extreme value of 5.32 obtained for one of the high consumers in the lowest discordance class (C). High consumers displayed mean *k* value of 1.15 and low consumers a mean value of 1.29 when this pair was excluded.

TABLE 36. Frequency of concordant and discordant pairs with respect to diabetic and borderline *k*-value ($k \leq 1.10$) in relation to alcohol consumption

		MZ			DZ			MZ+DZ		
		≤1	10>1	tot	≤1	10>1	tot	≤1	10>1	tot
HIGH ALC	≤1	10	1	6	11	20	31	11	26	37
	10>1		3	4	5	21	26	8	25	33
	tot		4	10	15	41	56	19	51	70

** $p < 0.01$

Ischemic heart disease

Investigations performed before 1965 (e.g. Hall *et al* 1953; Howell & Mannon 1960) on alcoholics with portal cirrhosis found at autopsy have revealed the presence of a thin vascular intima, particularly in the coronary arteries. After 1965 when non-cirrhotics were also included, an increasing number of reports began to be published on an association between excessive alcohol consumption and IHD (e.g. Sundby 1967; Pell & D'Alonzo 1973). As previously mentioned, the development of IHD is dependent on a genetic component. The purpose of this chapter is to describe the possible cardiovascular effects produced by longstanding use of alcoholic beverages when

inter-individual variations in genetic and early environmental factors may be assumed to be minimal. A number of cardiovascular diseases, symptoms and findings associated with IHD were the variables examined. The subject group and methods used are described in chapters I and II.

RESULTS

Overt IHD

None of the twins in the pooled zygosity group (MZ+DZ) had been treated for myocardial infarction, and only one reported severe chest pain lasting > 30 minutes (Table 39). He was one of the alcohol-negative twins. The prevalence of

TABLE 39 Cardiovascular diagnoses, findings and symptoms in relation to alcohol consumption.

	MZ (N=14 pairs)		DZ (N=36 pairs)		MZ+DZ (N=50 pairs)	
	High alc.	Low alc.	High alc.	Low alc.	High alc.	Low alc.
Myocardial infarction	0	0	0	0	0	0
Severe chest pain > 30 min.	0	1	0	0	0	1
Angina pectoris	4	2	4	3	8	5
Chest discomfort	1	4	13	13	14	17
Palpitations	0	0	14	5	14	5
Irregular heart rate	0	0	14	5	16	5
Slow heart rate	0	0	3	0	3	0
Intermittent claudication, suspected or confirmed	1	1	6	0	7	1
No or weak pulse in peripheral vessels	3	1	17	14	20	15
Apical systolic murmurs	7	7	30	25	37	32
Oedema of the ankles	0	1		1		
Cyanosis	1	0	3		4	
Xanthelasma	0	0	0	2	0	2
Arctus lipoides cornu	6	6	3	19	29	25
Diabetes mellitus	0	1	2	0	2	1

¹ Angina pectoris not included.
According to subjects' report.

TABLE 40 Frequency of concordant and discordant 11th respect 1 angina pectoris in relation to alcohol consumption.

		MZ				LOW ALC.				MZ+DZ			
		angina	chest	neg	tot	angina	chest	neg	tot	angina	chest	neg	tot
		discomfort				discomfort				discomfort			
HIGH ALC.	angina	1	1		4	1	2	1	4	2	3	3	8
	chest												
	discomfort	0	1	0	1	1	3	7	13	1	6	7	14
	neg	1	2	6	9	1	6	32	39	2	8	38	44
	total	2	4	8	14	3	13	40	56	5	17	48	70

angina pectoris (for definition see p. 19) was 9.3% in the subject group. No obvious difference was found between the high alcohol group (HAG) and the low alcohol group (LAG) in the prevalence of this symptom. Angina pectoris occurred in 8 and 5 subjects in the HAG and LAG respectively. It was present in one or both members of 11 pairs. Six of these pairs were concordant for angina pectoris or for chest discomfort (Table 40). Thus only 5 patently angina-discordant pairs could be found. These discordant pairs were almost equally distributed between the HAG and LAG. Chest discomfort, defined as chest pain not satisfying the criteria for angina pectoris, was reported by 14 and 17 subjects in the HAG and LAG respectively, 6 pairs being concordant. The results for angina pectoris and chest discomfort in both the MZ and DZ group were approximately similar.

Subjective heart symptoms

In the pooled zygosity group, there was an increased prevalence of subjective heart symptoms (palpitations, irregular heart rate, slow heart rate) for the high consumers of alcohol (Tables 39 and 41). Statistical differences were found in the incidence of irregular heart rates ($p < 0.01$) which might be due to arrhythmias, and palpitations ($p < 0.05$). The greatest disparity between the HAG and LAG twins was in the significantly larger number of combined subjective heart symp-

toms reported by the former group ($p < 0.01$).

























Peripheral circulation

The prevalence of clinical findings of impaired blood flow in the feet was approximately similar in the HAG and LAG in the pooled zygosity group (Tables 42 and 43). Suspected or manifest intermittent claudication was reported by 7 subjects in the HAG and 1 subject in the LAG respectively ($0.1 > p > 0.05$). Twenty twins of the HAG and 15 twins of the LAG had absent or only weak pulsations in the dorsal arteries of the feet on physical investigation. However suspected or manifest intermittent claudication was found in 6 of the HAG but in none of the LAG when the DZ group was analysed separately. This difference between the two groups is significant ($p < 0.05$). The fact that 17 HAG twins and 14 LAG twins in the DZ group had weak or absent pulsations in the dorsal pedal arteries provided no statistical support for the prevalence of suspected or confirmed intermittent claudication.

Other diagnoses, symptoms and findings associated with IHD

The prevalence of manifest diabetes mellitus in the subject group was about 11% (Table 39) which is about the average in a large population (Marble *et al.* 1971). The small number of subjects with this disease made it impossible to draw any conclusions. Differences between the high and

TABLE 41 *F* quantity of concordance and discordance with respect to subject heart symptoms in relation to alcohol consumption

		LOW ALC								
		MZ			DZ			MZ+DZ		
		pos	neg	tot	pos	neg	tot	pos	neg	tot
HIGH ALC	Palpitations	pos	0 	0	1 	13	14	1 	13	14
		neg	0 	14	4 	38	42	4 	52	56
		tot	0	14	5	51	56	5	65	70
	Irregular heart rate	pos	2 	2	2 	12	14	2 	14	16
		neg	0 	12	3 	39	42	3 	51	54
		tot	0	14	5	51	56	5	65	70
	Slow heart rate	pos	0 	0	0 	5	5	0 	5	5
		neg	0 	14	0 	51	51	0 	65	65
		tot	0	14	0	56	56	0	70	70
	All categories of subjective heart symptoms	pos	0 	2	2 	19	21	2 	21	23
		neg	0 	12	6 	29	35	6 	41	47
		tot	0	14	8	48	56	8	62	70

* = $p < 0.05$

** = $p < 0.01$

low alcohol consumers as regards findings such as cyanosis, oedema of the ankles or xanthelasma were negligible. Nor was the finding of systolic murmurs or arcus lipoides corneae, the prevalence of which were high, more common in the high than in the low alcohol group.

Radiographic examination of the heart

Disparity was found in the relative heart volumes (Jonzell 1939) in the highest discordance class (A) of the pooled zygoty group (Table 44). The mean volumes amounted to 366 and 337 ml for the HAG and LAG respectively ($p < 0.05$). The difference is certainly statistically significant but not consistent with the over all

pattern and is judged as having no clinical significance. The opposite trend was noted in the lower discordance classes (B and C).

Heart rate at rest

In the pooled zygoty group the HAG had a higher resting heart rate (Table 45) than the LAG ($p < 0.05$). The registered mean heart rates were 71.2 and 68.0 beats/min in the HAG and LAG respectively. Rising alcohol discordance did not increase this difference in pulse rate.

ECG findings at rest

The resting ECG in the pooled zygoty group disclosed a nonsignificantly higher number of

TABLE 42 Frequency of concordance and discordance with respect to expected or unformed intermuscular classification in relation to alcohol consumption

		LOW ALC											
		MZ			DZ			MZ+DZ					
		susp	pos	neg	tot	susp	pos	neg	tot	susp	pos	neg	tot
HIGH ALC	susp												
	pos	0	1	1		0	6*	6		0	7	7	
	neg	1	12	13		0	50	50		1	62	63	
	tot	1	13	14		0	56	56		1	69	70	

* $p < 0.05$

twins with left axis deviation (Table 46) in the high alcohol group than in the low alcohol group. This disparity was due to anterolateral bundle branch block in 3 of the 4 observed cases. One subject in the low alcohol group had Q waves not definitely diagnostic of previous infarction (Blackburn *et al* 1960). With respect to the ST segment at rest, 4 and 3 subjects were coded 4 1—3 (for definitions see p. 22) in the HAG and LAG respectively (Tables 46 and 47). The only subject in the study who had atrial fibrillation was found in the high alcohol group.

ECG during and after exercise

The number of twin pairs concordant and discordant for segmental ST depression during exercise in

relation to alcohol consumption is illustrated in Table 47. No differences in ST changes at maximal comparable work load were evident between the HAG and LAG twins in the pooled zygoty group. Of the 69 twin pairs whose response to exercise could be compared, 16 subjects in the high alcohol group and 13 in the low alcohol group were classified according to ST code 1—3. Six pairs were concordant according to this criterion. The prevalence of pathological ECG findings during maximal exercise was found to be somewhat higher in the MZ than in the DZ group. A reduced prevalence of ST changes was found in both the HAG and the LAG immediately after and 3 min after work. There were no ECG disparities between the high and low alcohol group

TABLE 43 Frequency of concordance and discordance with respect to or with palpation in dorsal pedal artery relation alcohol was ingested

		LOW ALC								
		MZ			DZ			MZ+DZ		
		pos	neg	tot	pos	neg	tot	pos	neg	tot
HIGH ALC	pos	1	2	3	3	14	17	4	16	20
	neg	0	11	11	11	28	39	11	39	50
	tot	1	13	14	14	42	56	15	55	70

TABLE 44 Relative heart volume in ml/m² BSA in relation to consumption

Class	Discordance in abs. alc/ year (grams)	MZ			DZ			MZ+DZ		
		High alc.	Low alc.	No. of pairs	High alc.	Low alc.	N of pairs	High alc.	Low alc.	No. of pairs
		M(SD)			M(SD)			M(SD)		
A	> 10,000	350(59)	361(68)	5	370(81)	332(47)	25	366(77)	337(49)	30
B	5,000—10,000	328(13)	384(71)	5	346(53)	365(61)	18	342(47)	370(63)	23
C	< 5,000	348(79)	363(67)	4	357(34)	352(34)	18	339(58)	355(55)	16
Total		341(52)	370(64)	14	355(67)	348(55)	55	352(64)	352(57)	69

= $p < 0.05$

Arrhythmias

No obvious differences in the prevalence of arrhythmias at rest were found between the HAG and LAG in the pooled syngosity group (Table 46). There was an increased incidence of arrhythmias in both alcohol groups during and after exercise (Table 48). A total of 13 subjects, all belonging to the DZ group, had arrhythmias classified into codes 1—6 in accordance with Table 48. No obvious disparities were found between the high and low alcohol consumers.

Comments

The most serious arrhythmia that occurred was a brief episode of ventricular tachycardia. This arrhythmia was seen in an alcohol-positive twin of the DZ group shortly after exercise and during continuous ECG registration. The arrhythmia spontaneously reverted to sinus rhythm, but the subject was moved immediately to the coronary

care unit. Since the serum enzyme values were not elevated, no new episodes of ventricular tachyarrhythmias occurred during continuous monitoring and no ECG changes suggestive of myocardial infarction appeared, he was permitted to leave hospital 24 h later.

DISCUSSION

The main finding in the present study was the absence of "hard IHD" i.e. verified myocardial infarction or death due to IHD (Keys *et al* 1972 b). Myocardial infarction is uncommon in young or middle-aged Swedish men (Brörck *et al* 1966, Tibblin 1967, Bengtsson 1973). Tibblin (1967) found a 1% prevalence of myocardial infarction in Swedish men aged 50. In Stamler's review (1973) Sweden was one of the industrialized countries with the lowest incidence of IHD death in the 25—64 age group. In Swedish males "hard IHD" (Keys *et al* 1972 b) occurs com-

TABLE 45 Heart rate at rest in relation to alcohol consumption (beats/min)

Class	Discordance in abs. alc/ year (grams)	MZ			DZ			MZ+DZ			f
		High alc.	Low alc.	No. of pairs	High alc.	Low alc.	No. of pairs	High alc.	Low alc.	N of pairs	
		M(SD)			M(SD)			M(SD)			
A	> 10,000	74.2(1.5)	75.6(1.5)	5	70.4(14.7)	66.6(13.0)	25	71.0(13.5)	68.1(12.5)	50	
B	5,000-10,000	68.6(9.2)	65.2(14.3)	5	69.6(15.5)	68.5(16.0)	18	69.4(14.2)	67.7(17.2)	23	
C	< 5,000	67.3(5.7)	71.5(13.6)	4	76.1(10.3)	67.2(11.5)	15	74.0(10.1)	68.2(11.8)	17	
Total		70.2(6.7)	70.7(11.8)	14	71.5(14.1)	67.4(13.5)	56	71.2(12.9)	68.0(13.1)	70*	

= $p < 0.05$

TABLE 46. ECG changes at rest in relation to alcohol consumption.

	MZ (N=14 pairs)		DZ (N=36 pairs)		MZ+DZ (N=70 pairs)	
	High alc.	Low alc.	High alc.	Low alc.	High alc.	Low alc.
Q wave pathological	—	—	—	1	—	1
Axis deviation, left	—	—	4	1	4	1
ST segment						
ST Code 1	—	—	1	—	1	—
2	—	—	1	—	1	—
3	—	—	2	3	2	3
4	—	1	1	—	1	1
5	5	4	8	5	13	9
6	—	—	—	—	—	—
7	—	—	1	—	1	—
T-wave						
> -3 mm	—	—	—	—	—	—
-1 to -3 mm	—	—	1	—	1	—
Flat or diphasic	—	—	1	—	1	—
Low amplitude	1	—	2	3	3	3
Anterolateral bundle branch block	—	—	3	1	3	1
Sinus tachycardia	—	—	2	2	2	2
Arrhythmias:						
Atrial fibrillation	—	—	1	—	1	—
Frequent multifocal VEB	—	—	—	—	—	—
Frequent unifocal VEB	—	—	—	—	—	—
Occasional multifocal VEB	—	—	—	—	—	—
Occasional unifocal VEB	—	—	—	—	—	—
(2-4 in 20 complexes)	—	—	—	—	—	—
Occasional VEB	1	—	2	1	3	1
Frequent SVEB (> 3 in 20)	—	—	—	1	—	1
SVEB (2-4 in 20 complexes)	—	—	—	—	—	—
Occasional SVEB	—	—	—	—	—	—

mostly at an advanced age. Since none of the investigated twins had suffered from a myocardial infarction, the 10 deceased twins in the subject group were also traced (p. 15). The death of three in the high alcohol group and one in the low alcohol group proved to be associated with IHD.

Thirteen persons (9.3%) in the entire subject group were angina pectoris-positive, with a slightly higher prevalence found in the high (11.5%) alcohol group than in the low (7.1%) alcohol group. The number of positive answers was somewhat higher than the number usually reported. In his study of smoking-discordant twins Lundman

(1966) found angina or suspected angina in 7% of his subjects. However, the average age was about 50 in his investigation as compared to 55 years in the group presented here. Rose (1968) found that the prevalence of angina pectoris was relatively constant at approximately 4% by repeated assessment over a 4-year period of a sample of 1,136 men initially aged 35-59. The large majority were positive on only a single occasion and the 4-year period prevalence was 10.4%.

It was of interest to note in the present study that angina pectoris was not less prevalent in the high than in the low alcohol group. Earlier

TABLE 47 Frequency of concordance and discordance in respect to ST segment depression at rest and during maximal comparable work in relation to alcohol consumption

		LOW ALC								
		MZ			DZ			MZ+DZ		
		ST-code	1-3	0 or tot 4-7	1-3	0 or tot 4-7	1-3	0 or tot 4-7	1-3	0 or tot 4-7
HIGH ALC	At rest	1-3	0	0	1	4	1	4		
		0 or 4-7	0	14	2	50	2	64		
		tot	0	14	3	53	3	67		
	Max comp work	1-3	2	4	4	12	6	16		
		0 or 4-7	1	9	6	43	7	53		
		tot	3	14	10	55	13	69		

questionnaire-studies of the Swedish and U.S. twin registries have shown that the positive members of pairs discordant with respect to alcohol consumption had significantly increased rates of angina pectoris among both DZ and MZ twins (Twin Registries in the study of chronic diseases 1971). Alexander (1966) suggested "that angina is conspicuously absent in alcoholics. This may well be true of alcoholics but not of subjects whose consumption of alcohol is only moderately excessive.

Many authors have stressed that angina pectoris is a fairly unreliable symptom of IHD (e.g. Lundman 1966, Lundman *et al* 1971, Raftery *et al* 1971, Keys *et al* 1972 b). Rose (1961) has shown that respiratory symptoms in smokers may result in a false positive diagnosis of angina pectoris. This might influence assessments of the prevalence of angina to some extent, especially among high alcohol consumers who were found to be heavy consumers of tobacco also (chapter IV).

The present interview results showed that subjective heart symptoms (palpitations, irregular heart rhythm and slow heart rate) occurred more frequently among high than among low alcohol consumers. These results were not verified by ECG

during and after exercise. However the subjective symptoms reported above should not be set aside as the subjective reports refer to many years of observation as compared to a single ECG record. Earlier studies have disclosed link between high alcohol intake and cardiac arrhythmias (Fredrikson & Hed 1938, Jørgensen & Hedebo 1968, Carlsson 1970). Vetter *et al* (1967) found a correlation between the presence of cardiac arrhythmias and subnormal serum potassium in acute alcohol withdrawal. A low serum potassium level in alcoholics was also reported by Johansson and a direct toxic action by alcohol on the heart symptoms in the alcohol positive group could be increased secretion of adrenaline after ingestion of alcohol (Perman 1962, Broholt *et al* 1970) and a direct toxic action by alcohol on the heart (Alexander 1966, Brandfoerbrener 1967). James & Bear (1967) showed that acetaldehyde which is a metabolite of alcohol, had a direct effect on the sinus node, mainly due to its noradrenaline-releasing effects.

The results point to a link between alcohol consumption and resting heart rate. In any case, this variable did not discriminate strongly between the high and low alcohol group in the present study

TABLE 48 Frequency of concordance and discordance with respect to total duration of arrhythmias during and after exercise in relation to alcohol consumption

		MZ			DZ			MZ+DZ		
code		1-6	0 or 7-10	tot	1-6	0 or 7-10	tot	1-6	0 or 7-10	tot
HIGH ALC	1-6	0	0	0	1	4	5	1	4	5
	0 or 7-10	0	14	14	7	43	50	7	57	64
	tot	0	14	14	8	47	55	8	61	69

Code

- No arrhythmia
- 1 Ventricular tachycardia
- 2 Atrial fibrillation
- 3 Frequent (more than 5 out of 20) multifocal ventricular premature beats
- 4 Frequent (more than 5 out of 20) unifocal ventricular premature beats
- 5 Multifocal (2 to 4 out of 20) ectricular premature beats

- 6 Unifocal (2 to 4 out of 20) ectricular premature beats
- 7 Occasional ectricular premature beats
- 8 Frequent (more than 5 out of 20) supra ectricular (including nodal) premature beats
- 9 Supraventricular (including nodal) premature beats (2 to 4 out of 20)
- 10 Occasional supraventricular (including nodal) premature beats

In the case of more than one type of arrhythmia, each subject was only coded once in accordance with the lowest code number

Mild tachycardia is usually encountered in studies of alcoholics (Fredriksen & Hed 1958, Evans 1959, Birgden & Robinson 1964, Carlsson 1970, German 1973). Insurance data have suggested a relationship between causal determinations of resting heart rate and subsequent mortality (Stemler 1973). Berkson *et al* (1970) reported that a heart rate of > 80 beats per minute was associated with higher 10-year IHD mortality rates. On the other hand, Blackburn *et al* (1970) was unable to find any excess 5 year IHD incidence in initially IHD-free subjects with resting heart rates of more than 100 beats/minute.

Radiographically determined heart volume displayed significant disparity in the highest discordance class of the DZ group. This result was not confirmed in any other subgroup and is not considered to be of clinical importance. It is surprising that the link between alcohol consumption

and relative heart size was not more pronounced. Even though most of the twins in the high alcohol group were moderate alcohol consumers, a few subjects had a consumption adequate for the development of cardiomyopathy (Evans 1957, Birgden & Robinson 1964, Brandfonbrener 1967). Hypertension may be another possible reason for a relatively enlarged heart volume (Huzarfelt 1963, Tibblin 1967). This condition was found to be more prevalent in the alcohol-positive group (chapter V).

Electrocardiography during and after exercise is regarded as a fairly reliable method for the detection of subclinical IHD (Blomqvist 1965, 1971, Astrand 1973, Helfant *et al* 1973). This method also increases the possibility of a proper evaluation of arrhythmias (Konowsky *et al* 1971). Since it has been shown (Blomqvist 1965) that the magnitude and frequency of ST segment depression in

exercise is directly related to the relative workload, it is important to keep this sufficiently heavy. The subjects in the present study were asked to continue exercising "as long as possible, and so high workloads were obtained (average for the entire subject group 140 Watts). Thus, few affected subjects are assumed to have passed through the investigation without IHD being detected.

The workload for each subject of a pair must be about equal if the intra-pair comparison is to be valid. Thus the ECG records made during exercise were interpreted at the highest comparable heart rate, i.e. the highest heart rate recorded by the twin with the lowest final heart rate of a pair.

Follow-up studies of asymptomatic subjects have demonstrated that a horizontal ST depression, especially during or after exercise (Mathugly 1962, Blomqvist 1965, Mason *et al* 1967, Astrand 1973) is associated with a high risk of the development of clinical IHD. In a 10-year follow-up study of about 1 000 subjects, Mattingly (1962) showed that the incidence of IHD was about 8 times higher after 10 years in subjects with ischemic ST depression after exercise than in subjects without this. Mason *et al* (1967) found a 81% sensitivity for positive ST changes in comparison with significant changes in coronary arteriography. According to Blomqvist (1971) the prognostic significance of a pathologic exercise ECG appears to be independent of other known risk factors. ECG studies of alcoholics have shown that the most commonly encountered findings are mild tachycardia, extrasystoles, atrial fibrillation and conduction disturbances (Fredriksen & Hed 1958, Evans 1959, Bridgen & Robinson 1964, Carlsson 1970, German 1973). Evans (1959) identified T wave deformation of the ECG as a distinctive feature. Levine *et al* (1965) and Priest *et al* (1966) demonstrated that the deformed T wave is a good diagnostic criterion of alcoholic cardiomyopathy. Since practically none of the alcohol-positive twins showed T wave changes, it seems reasonable to assume that few of the sub-

jects had any cardiomyopathy. This statement is also supported by the X-ray findings indicating that relative heart volumes were essentially the same for the high and low alcohol consumers. In addition, the clinical examination provided no basis for the diagnosis of cardiomyopathy.

No obvious differences were found between the high and low alcohol consumers with respect to IHD judged on the basis of clinical or ECG findings at rest and during exercise. Thus, these results do not point to an association between long lasting moderate consumption of alcohol and IHD or cardiomyopathy in males when the influence of genetic and early environmental factors was assumed to be minimal. The results could possibly be ascribed to the fact that the examined twins were too young for detection of IHD using the methods applied, and to the influence of genetic factors.

SUMMARY

Seventy alcohol-discordant twin pairs were investigated with respect to symptoms, signs and diagnoses associated with IHD. None of the twins had suffered from myocardial infarction, and the prevalence of angina pectoris was essentially the same in the high and low alcohol groups. There is no evidence indicating that any of the subjects had cardiomyopathy. In the pooled zygosity group (MZ+DZ) reported subjective heart symptoms, such as palpitations and sensations resembling arrhythmias, appeared more frequently in the high rather than in the low alcohol consumers. A weak significance was also found for this group as regards elevated heart rate and as regards a slight increase in relative heart volume in the highest discordance class. The ECG at rest and during exercise showed no significant difference between the high and low alcohol consumers. It may be concluded that there is no apparent association between alcohol consumption and IHD when the influence of genetic and early environmental factors is minimal.

General discussion

Studies of the possible somatic effects of alcohol consumption have usually employed subjects who were obvious alcoholics. IHD investigations prior to 1965 dealt mainly with alcoholics with cirrhosis of the liver, a disorder seldom associated with atherosclerosis, but with an unfavourable prognosis. Epidemiological studies have shown that subjects with an excessive consumption of alcohol are more prone to IHD than the rest of the population. Furthermore, non-vagrant alcoholics have been reported to be more prone to IHD than vagrant subjects (Sundby 1967). It is evident that moderately excessive alcohol consumers, gamfully employed and apparently well adapted socially have seldom been included in earlier studies. These subjects represent the vast majority of alcohol consumers. It was thus considered of major interest to investigate the possible effect of this habit on the appearance of factors associated with IHD subclinical as well as manifest disease.

It is well known that a subject's own account of his alcohol consumption is often biased due to his inclination to reply in accordance with social desirability. In the present study the classification of alcohol consumption is considered reliable. On reassessing each twin's consumption 1972/73 as compared to 1967 it was found constant relative to his co-twin. Reproducibility was thus 100 %. The following criteria of validity were studied in detail: specific anamnestic variables, physical findings and serum enzymes associated with a high alcohol intake. These items disclosed significant disparities between high and low alcohol consumers, with the exception of findings indicative of more serious somatic damage, such as jaundice, ascites and pathological serum enzymes. The results, including these exceptions, confirmed the presence of moderate alcohol consumption in the high alcohol group and that the classification of alcohol ingestion must be considered valid.

The role of hereditary factors in various diseases should be taken into consideration more often than has been the case, especially when studying the etiologic importance of an environmental factor in the development of disease. Since hereditary factors have been found to play an important role in both drinking habits and in the development of IHD it was considered important to minimize their influence, as far as possible, by investigating a group of alcohol-discordant twins. The twin method means that the influence of genetic factors is minimized and that differences in early environment are also to a great extent reduced. In addition, perfect age matching is obtained.

Using monozygotic twin pairs, the influence of genetic factors is completely eliminated. Furthermore, genetic factors are shared to about 50 % in a pair of same-sexed dizygotic twins. In the present study results were mainly derived from the dizygotic series. In the pooled zygosity group (MZ+DZ) however the influence of genetic factors was eliminated to 60 % due to the fact that the study group comprised 14 MZ and 56 DZ twin pairs. The present study satisfactorily reduced the influence of genetic factors in comparison with conventional studies of probands and control subjects.

Thirteen of the initial 92 pairs were lost due to death (8 pairs) or severe illness. The cause of death in relation to previously reported alcohol consumption was ascertained. The number of pairs investigated (70) deviated only slightly from the target group (79 pairs). This must be considered satisfactory as many of the subjects had to travel more than 500 miles in order to reach the examiner. Nine pairs refused to participate or were unable to take part but all were contacted by telephone. The loss of a pair was often due to one of its members being unable to make the journey because of his intemperate habits. So these losses

most probably reduced the possibility of showing differences between the groups.

Even though the Swedish Twin Registry contains a large material, only 86 potentially alcohol-discordant twin pairs could be found. This was because of the criteria for selection, age, sex and alcohol discordance. Moreover the twins showed a high rate of concordance with respect to intra pair drinking habits. A carefully devised clinical study was still considered of value in showing whether these alcohol-discordant twin pairs differed regarding factors associated with IHD subclinical as well as manifest disease. In evaluating the results of the present study the difficulties arising from the relatively limited number of subjects making especially the negative statements inconclusive, must be conceded. The difference between stating that "an effect is absent" and that "an effect can not be demonstrated" is obvious.

It is evident from the present study that moderate, longstanding alcohol consumption is associated with other factors known to be related to the development of IHD. An obvious link was recorded as regards smoking. It is well known that alcohol consumption is associated with this habit. So the problem must be faced whether the results in the present study can be explained by smoking alone. That this is not so is evident from the following table:

It is apparent that factors known to be associated with the development of IHD are present at higher levels in the high alcohol group than in the low alcohol group even when the effect of smoking is eliminated. In a study of smoking-discordant twin pairs, Lundman (1966) was able to disclose an inverse relationship between smoking and blood pressure, weight, serum cholesterol and serum triglycerides. Using a multiple logistic model on a random sample, Wilhelmsen *et al* (1973) demonstrated the absence of any correlation between smoking and blood pressure or serum lipids. In an epidemiological study of asymptomatic hyperuricemics, Fessel *et al* (1973) was unable to show that cigarette smoking had any significant effect on serum uric acid. The same has also been suggested with respect to intravenous glucose tolerance (Wahlberg 1966). So, the metabolic effects in humans of even moderate doses of alcohol are considerable and may be compared to an extreme exposure to tobacco. Except for the induction of symptoms such as cough, phlegm, asthma, and dyspnea, the higher daily cigarette consumption in the high alcohol group can not explain the increased prevalence of factors associated with IHD.

It is well known that many of the metabolic variables, e.g. fasting blood glucose and serum uric acid, are related to other variables, such as

Examined variables in relation to alcohol consumption in 111 twin pairs discordant with respect to smoking

	MZ+DZ (N=11 pairs)		
	High alc	Low alc	
	M(SD)		
Casual systolic blood pressure	151 (15)	147 (19)	mm Hg
Basal systolic blood pressure	93 (11)	89 (9)	mm Hg
Absolute weight	72 (12)	68 (9)	kg
Serum cholesterol	242 (37)	232 (44)	mg/100 ml
Serum triglycerides	129 (45)	148 (72)	mg/100 ml
Serum uric acid	5.8 (11)	5.2 (8)	mg/100 ml
Fasting blood glucose	83 (21)	81 (10)	mg/100 ml
IVGTT (k value)	5	2	

Number of subjects with borderline or pathological values

serum cholesterol and blood pressure, in a complicated metabolic network (Epstein 1973a). The present investigation suggests that long-standing moderate alcohol consumption has a number of physiological and metabolic effects. Thus, systolic and diastolic blood pressures, fasting blood glucose and serum uric acid were significantly higher in the high alcohol group than in the low. Significant differences were also found for intravenous glucose tolerance and the intra-pair level of serum cholesterol, the high consumers showing the higher number of pathological or elevated values. That elevated and even pathologically high levels are confined to the high alcohol group is striking.

As the present investigation was performed after at least 12 h of fasting no alcohol was consumed by the twins from the evening before. That this was so in all cases but one was verified by means of a blood alcohol assay. Casual measurements of metabolic variables such as blood glucose, serum lipids, serum uric acid etc., have been performed in some large population studies. Results from such investigations might partly be explained by the acute effects of alcohol. Nevertheless, such casual measurements are of great value, as they most probably give a truer picture of the subject's normal condition.

Sensitive methods are important when conclusions are to be drawn regarding IHD. In the present study the resting ECG was supplemented by an exercise ECG, a method considered fairly reliable in the detection of IHD (e.g. Blomqvist 1971, Astrand 1973). The value of the ECG during exercise was further enhanced as all subjects but one were able to carry out the test, in most cases at a submaximal level.

None of the twins had suffered from myocardial infarction. The prevalence of angina pectoris was higher but not significantly so in the high alcohol group than in the low. The ECG at rest or during exercise did not differ between the twin groups. Thus, no differences could be demonstrated between the high and low alcohol consumers with regard to manifest or subclinical IHD. This may be explained by the small size of the sample by genetic factors, and by the methods available for the detection of IHD being comparatively crude for intra-pair comparison. Another possible explanation is the low mean age of the sample: the twins examined might still be too young to have developed IHD. Therefore a follow-up of the present subject group will be performed.

General summary and conclusions

The main purpose of this study was to investigate if there is a relationship between alcohol consumption and factors associated with IHD subclinical as well as overt disease, in apparently healthy gainfully employed men when the influence of genetic and early environmental factors was minimal.

Anamnestic variables, somatic findings and some serum enzymes associated with a high alcohol intake were also studied, mainly in order to establish that the selected twin sample really consisted of moderately alcohol-discordant twin pairs. Significant differences were obtained between the twins for the following anamnestic variables: blackouts, eyeopeners, intoxication, heavy consumption at parties, "more than half a bottle on the same occasion" and subjective classification of personal alcohol habits. In addition, the alcohol positive group showed a significantly increased prevalence of hepatomegaly tremor vascular spiders—paper money skin and Dupuytren's contracture but not of more serious findings, such as jaundice and ascites. Serum enzymes also showed significant disparities for SGOT, SGT and SALP the slightly higher but almost consistently nonpathological values being found among the high alcohol consumers. On the basis of these results and the calculations of alcohol consumed per year the twins were designated moderately alcohol discordant and assigned to a high alcohol group (HAG) and a corresponding low alcohol group (LAG).

The material comprised 70 pairs, 14 monozygotic and 56 dizygotic same sexed male twin pairs, aged 45—65 years. They made up 76 % of a sample selected from the Swedish Twin Registry containing some 4 500 male pairs, compiled at the Department of Environmental Hygiene, Karolinska Institute, and the National Institute of Public Health, Stockholm. The chief selection criterion

was discordance with respect to alcohol consumption set at $> 10\,000$ and $< 2\,000$ g of absolute alcohol/year.

A sociological and medical history was taken using a standardized interview. The cardiovascular questions were designed by the London School of Hygiene and Tropical Medicine. The zygosity classification was obtained by serological determinations carried out at the National Laboratory of Forensic Medicine in Stockholm.

The subjects were submitted to an examination, including anthropometric and blood pressure measurements, radiographic examination of the chest and an electrocardiographic examination before, during and after a maximal exercise test. Blood samples were drawn after 12 hours of fasting and analysed for serum cholesterol, serum triglycerides, blood glucose, serum uric acid and alcohol in blood. In addition an intravenous glucose tolerance test and lipoprotein electrophoresis of serum were performed.

The hypotheses stated in the present study (page 12) were confirmed in the pooled zygosity group (MZ+DZ) in respect to the following findings:

1 Smoking was found slightly more often in the high alcohol group. Pipe and cigar smoking was significantly more frequent among the low consumers of alcohol. Quantitative cigarette consumption showed the most striking differences, and subjects in the high alcohol group smoked significantly more cigarettes daily. These differences applied to present smokers but were also applicable when former smokers were included. Significant disparities in the prevalence of pulmonary symptoms, such as cough, phlegm, dyspnea, and asthma, were found: all these symptoms were more common in the high alcohol group.

2. A greater number of the high alcohol consumers than the low had previous history of hypertension. A significant link between alcohol consumption and current blood pressure level was also obtained. This was true of both systolic and diastolic pressures. The findings were independent of whether measurement was casual or basal.

3. No significant association was found between alcohol consumption and any weight parameter. The alcohol-positive twins in the highest discordance class had a significantly thicker layer of subcutaneous fat in the triceps area than their low consumption co-twin. No disparities were found for the subscapular area.

4. Small and almost consistently non-significant differences were found in serum lipids. No evident disparities were observed in mean values for serum cholesterol, triglyceride levels, and serum lipoproteins. A significantly greater proportion of the high alcohol consumers exceeded the > 200 mg/100 ml limit for serum cholesterol in the intra-pair evaluation.

5. A significant association was found between alcohol consumption and fasting blood glucose, and significantly more of the high consumers had fasting blood glucose values > 100 mg/100 ml. Significantly lower k values were noted for intravenous glucose tolerance among high consumers. The prevalence of "borderline" or diabetic k -values was 53 % in high alcohol consumers and 27 % in low alcohol consumers.

6. Significantly higher levels of serum uric acid were found in the high alcohol group. The greatest difference was obtained in the highest discordance class. Serum uric acid ≥ 8 mg/100 ml was also significantly more common in the high alcohol group.

7. There was no excess morbidity owing to overt

IHD or cardiomyopathy among the high alcohol consumers. None of the twins had suffered from myocardial infarction, and the prevalence of angina pectoris was higher but not significantly so, in the high than the low alcohol group. The prevalence of subclinical IHD or arrhythmias, as shown by the ECG at rest or during exercise was the same for both groups. A significantly greater number of the twins in the high alcohol group than in the low reported subjective heart symptoms, such as palpitations and sensations which could be described as arrhythmias.

CONCLUSIONS

On the basis of the present study significant differences were established for moderately alcohol-discordant male twin pairs, aged 45—65 years, for systolic and diastolic blood pressures, high cigarette consumption, phlegm, cough, asthma, dyspnea, serum cholesterol values > 200 mg/100 ml, hyperuricemia, elevated fasting blood glucose and diabetic k values; the greater number of findings or pathological values were found in the high alcohol consumption compared to the low alcohol consumption co-twins. No conclusion can be drawn with respect to overt or subclinical IHD while statistically significant disparities were found with regard to subjective heart symptoms, such as palpitations and sensations possibly indicative of arrhythmias.

Despite obvious differences in the prevalence of number of conventional risk factors, no disparity has yet been found in that of manifest IHD between subjects with high or low alcohol consumption. This finding may well be due to the limited size of the subject group, to the low prevalence of IHD anticipated in a group with such a low mean age, or to its constitutional similarity

29. BJÖRCK, G. & BLOMQUIST, G. & SIEVERS, J. Cholesterol values in patients with myocardial infarction and in a normal control group. *Acta Med Scand* 156 493, 1957
30. BJÖRCK, G. BLOMQUIST, G. HALL, P. JOHANSSON, B. W. LARSSON, E., MALMÖRS, H. SIEVERS, J. & SWAHN, H. Coronary heart disease in a population in the South of Sweden. *Acta Med Scand* 179-663 1966.
31. BJÖRCK, G. BOLANDER, A. M. & GYLLENHÄMMAR, K. Dödsfall i ischemisk hjärtsjukdom i Sverige. *Läkertidningen* 67 3393, 1970
32. BJÖRKMAN, N. M. En jämförelse mellan rekord och intervjuer vid samling av data om alkoholbruk. Svenska Folkets Alkohol åren 50-71 77 43, 1971.
33. BLACKBURN, H., KEYS, A., SIMONSON, E., RAUTAHARJU, J. & PUNSAAR, S. The electrocardiogram in population studies. A classification system. *Circulation* 21 1160, 1960.
34. BLACKBURN, H. TAYLOR, H. L. & KEYS, A. The electrocardiogram in prediction of first year coronary heart disease incidence among men aged forty through fifty-nine. *Circulation suppl.* 1 1970
35. BLOMQUIST, G. The Frank lead exercise electrocardiogram. A quantitative study based on averaging technique and digital computer analysis. *Acta Med Scand suppl.* 440 1965
36. BLOMQUIST, G. Use of exercise testing for diagnostic and functional evaluation of patients with arteriosclerotic heart disease. *Circulation* 44 1120, 1971
37. BRANDFONBRENER, M. Alcoholism and the heart. *J Chron Dis* 20 345 1967
38. BRECKENRIDGE, A. Hypertension and hyperuricemia. *Lancet* 1 15 1966.
39. BRIGDEN, W. & ROBINSON, J. Alcoholic heart disease. *Br Med J* 2 1283, 1964
40. The British Medical Research Council Committee on the Aetiology of Chronic Bronchitis. Standardized questionnaire on respiratory symptoms. *Br Med J* 2 1665 1960
41. BROHULT, J. CARLSON, L. A. & REICHARD, H. Serumenzyme activities, cholesterol, and triglycerides in serum after intake of alcohol. *Scand J Clin Lab Invest Suppl.* 92 82, 1966.
42. BROHULT, J. LEVI, L. & REICHARD, H. Urinary excretion of adrenal hormones in man. Effects of ethanol ingestion, and their modification by chlormethazolo. *Acta Med Scand* 188 5 1970
43. BURCH, G. E. & DePASQUALE, N. P. Editorial. Alcoholic lung disease—an hypothesis. *Am Heart J* 73 147 1967
44. BURGESS, M. FEJFAR, Z. & KAGAN, A. R. Arterial hypertension and ischaemic heart disease: comparison in epidemiological studies. 5. Work in progress. *WHO Chron* 17 97 1965
45. CABOT, R. The relation of alcohol to arteriosclerosis. *JAMA* 43 774 1904
46. CARLSON, L. A. Serum lipids in men with myocardial infarction. *Acta Med Scand* 167 399 1960
47. CARLSON, L. A. & BÖTTIGER, L. E. Ischaemic heart disease in relation to fasting values of plasma triglycerides and cholesterol. Stockholm prospectiv study. *Lancet* 1 865 1972.
48. CARLSSON, C. Alkoholism som sjukdom. *Scand U Books* Stockholm 1970
49. CARRIE, J. R. G. Finger tremor in alcoholic patients. *J Neurol Neurosurg Psychiatry* 28 329 1965
50. CEDERLÖF, R. The twin method in epidemiological studies on chronic disease. ESSELTZ, Stockholm 1966.
51. CEDERLÖF, R. FRIBERG, L., JONSSON, E. & KAIJ, L. Studies on similarity diagnosis in twins with the aid of mailed questionnaires. *Acta Genet* 11 338, 1961
52. CEDERLÖF, R., FRIBERG, L., JONSSON, E. & KAIJ, L. Respiratory symptoms and angina pectoris in twins with reference to smoking habits. *Arch Environ Health* 13 726, 1966
53. CEDERLÖF, R., FRIBERG, L. & JONSSON, E. Hereditary factors and angina pectoris. *Arch Environ Health* 14 397 1967
54. CEDERLÖF, R. FLODERUS, B. & FRIBERG, L. The Swedish Twin Registry Past and future use. *Acta Genet Med Gemellol* 19 551 1970
55. CERASI, E. Plasma insulin response to glucose infusion in healthy subjects, patients with diabetes mellitus and subjects with genetic prediabetes. *Acta Endocrinol* 41 (Thesis no 8) 1967
56. CERASI, E. & LUFT, R. Insulin response to glucose infusion in diabetic and non-diabetic monosymptomatic twins pairs. Genetic control of insulin response. *Acta Endocrinol* 35 330 1967
57. CHAIT, A., MANCINI, M. FEBRUARY, A. W. & LEWIS, B. Clinical and metabolic study of alcoholic hyperlipidaemia. *Lancet* 2 62 1972.
58. COBB, S. KING, S. & CHEN, H. Differences between respondents and non-respondents in a morbidity survey involving clinical examination. *J Chron Dis* 6 95 1957
59. COCHRANE, H. Depyriten contracture. *Lancet* 2 594, 1972
60. CUTSHALL, B. J. The Saunders-Sutton syndrome: An analysis of delirium tremens. *Q J Stud Alcohol* 26 423 1965

61. D'ALONZO C. A. & PELL, S. Cardiovascular disease among problem drinkers. *J Occup Med* 7 344, 1968.
62. DAVIES, K. M. The influence of alcohol on mortality. *Proceeding of the Home Office Lfj Underwriter As nat Meeting* 46 159 1963
63. DAWBER, T. R. & KANNEL, W. B. Atherosclerosis and your pathogenetic implications from epidemiologic observations. *J Am Ger Soc* 10 803 1962.
64. DENCKER, S. J. A follow-up study of 128 closed head injuries in twins using co-twins as controls. *Acta Psych Neurol Scand* suppl 123, 1958.
65. DEUTSCHER, S. EPSTEIN F. H. & KJELBERG, M. O. Familial aggregation of factors associated with coronary heart disease. *Circulation* 33 911, 1966
66. DOLLERY C. T. DUNCAN H. & SCHUMER, H. Hyperuricaemia related to treatment of hypertension. *Br Med J* 2 832, 1960.
67. DORNHORST A. & OUYANG, A. Effect of alcohol on glucose tolerance. *Lancet* 2 937 1971
68. DOWNE, W. W. BOYLE, J. A. GREIG W. R., BUCHANAN W. W. & ALEPA, F. P. Relative roles of genetic and environmental factors in control of blood pressure in normotensive subjects. *Br Heart J* 31 21, 1969
69. DUBLIN L. The influence of weight on certain causes of death. *Human Biol* 2 159 1930
70. DUNDEE, J. W. Alcohol and glucose tolerance. *Lancet* 2 433, 1972
71. ECKERSTROM, S. Clinical and prognostic aspects of acute coronary occlusion. *Acta Med Scand* 139 84, 1951
72. EDMONDSON H. A., HALL, H. M. & MYERS, R. O. Pathology of alcoholism. *Alcoholism* Ed by G. N. Thompson, 1956 C. C. Thomas Publ Springfield, Ill. USA.
73. EPSTEIN H. H. Hereditary aspects of coronary heart disease. *Am Heart J* 67 443 1964.
74. EPSTEIN F. H. The epidemiology of coronary heart disease. A review. *J Chron Dis* 18 733, 1965
75. EPSTEIN, F. H. Multiple risk factors and the prediction of coronary heart disease. *Bull NY Acad Med* 44 916, 1968
76. EPSTEIN, F. H. Elevated blood sugar. The associated cardiovascular risk. *Miss Med* 52 1271, 1969
77. EPSTEIN, F. H. Glucose intolerance and cardiovascular disease. *Trieste* 12 3 1973
78. EPSTEIN, F. H. Coronary heart disease epidemiology revisited. Clinical and community aspects. *Circulation* 48 183, 1973.
79. ENTMACHER, F. S. ROOT H. F. & MARKS, H. H. Longevity of diabetic patients in recent years. *Diabetes* 13 373, 1964.
80. ERIKSSON, K. Factors affecting voluntary alcohol consumption in the albino rat. *Ann Zool Fennici* 6.227 1969
81. EVANS, W. The electrocardiogram of alcoholic cardiomyopathy. *Br Heart J* 21 443 1959
82. FEJFAR, Z. "Risk factors in ischemic heart disease. *Acta Cardiol* suppl. 13 7 1972.
83. FESSEL, W. J. SIEGELAUB A. B. & JOHNSON, E. S. Correlates and consequences of asymptomatic hyperuricaemia. *Arch Intern Med* 132 44, 1973
84. FISHER, R. A. Cancer and smoking. *Nature* 182 396, 1958.
85. FOLIN, O. Standardized methods for determination of urea acid in unaltered blood and virus. *J Biol Chem.* 101 111, 1933.
86. FREDRIKSEN, P. & HED R. Clinical studies in chronic alcoholism. III. Cardiac changes in chronic alcoholism. An electrocardiographic study. *Acta Med Scand* 162.203, 1958.
87. FREDRIKSON D. S. & LEES, R. S. A system for phenotyping hyperlipoproteinaemia. *Circulation* 31 321, 1965
88. FRIBERG, L., KAJI, L., DENCKER, S. J. & JOHANSSON E. Smoking habits of monozygotic and dizygotic twins. *Br Med J* 1 1090 1959
89. FRIBERG, L., CEDERLOF R. LUNDMAN, T. & OLSSON, H. Mortality in smoking discordant monozygotic and dizygotic twins. *Arch Environ Health* 21 508 1970.
90. FRIBERG, L., CEDERLOF R. LORICH, U. LUNDMAN T. & deFAJRE, U. Mortality in twins in relation to smoking habits and alcohol problems. *Arch Environ Health* 27 294, 1973
91. FRISK, A. R., WERKO L., HOLMGREN, A. & STROM, G. Stockholm city health survey 1934. *Acta Med Scand* 163 1 1939
92. GEDDA, L. Tales in history and science. C. C. Thomas Springfield, Ill., 1961
93. GERMAN E. Medical problems in chronic alcoholic men. *J Chron Dis* 26 661 1973.
94. GERTLER, M. M. GARN S. M. & LEVINE, S. A. Serum urea acid in relation to age and physique on health and in coronary heart disease. *Ann Intern Med* 34 1421, 1971
95. GERTLER, M. M. & WHITE, P. D. Coronary heart disease in young adults. *Harvard University Press Cambridge, Mass* 1954
96. GESELL, A. The method of co-twin control. *Science* 93 446, 1942.
97. GLASS, H. B. Chapter XXII Genetic aspects of adaptability. *Res. Publ As Res Nerv Ment Dis* 33 367 1954.

98. GOFMAN, J W The blood pressure factor in coronary heart disease. C. C. Thomas Springfield, Ill., 1959
99. GOLDBERG, L. & RYDBERG U. Automated enzymatic microdetermination of ethanol in blood and urine. Presented at the Technicon Fifth International Symposium *Automation in Analytical Chemistry* Oct. 1965
100. GOLDSTEIN J L, HAZZARD W R, SCHROTT H G, BIERMAN E L & MOTULSKY A G Hyperlipidemia in coronary heart disease. I. Lipid levels in 500 survivors of myocardial infarction. *J Clin Invest* 52 1553 1973
101. GOTTLIEB, M. S. & ROOT H. F. Diabetes mellitus in twins. *Diabetes* 17:693 1968
102. GRANT W C, WASSERMAN F, RODENSKY F L & THOMSON R. V. The incidence of myocardial infarction in portal cirrhosis. *Acta Intern Med* 51 774 1959
103. GREEN G M & KASS, E. H. The influence of bacterial species on pulmonary resistance to infection in mice subjected to hypoxia, cold, stress, and ethanolic intoxication. *Br J Exp Pathol* 46 360 1965
104. GROLLMAN A. The influence of alcohol on the circulation. *Q J Stud Alcohol* 33 1942
105. GUSTAFSON, A. Serum lipoproteins in coronary heart disease. The possibility of prediction, p 201, *Skandia Intern symposia*. Nord. Bokhandeln, Stockholm 1973
106. HALL, E. M., OLSEN A. Y. & DAVIS, F. E. Portal cirrhosis. Clinical and pathologic review of 782 cases from 16,600 necropsies. *Am J Pathol* 29 993, 1953
107. HAMMOND E. C., GARFINKEL, I. & SEIDMAN H. Longevity of parents and grandparents in relation to coronary heart disease and associated variables. *Chirobiol* 43 31 1971
108. HANSEN, O. E. Hyperurcemia in cerebral infarction. *Acta Neurol Scand* 41 357 1965
109. HANSEN O. E. Hyperurcemia, gout, and atherosclerosis. *Am Heart J* 72 570, 1966
110. HARVALD B. & HAUGE, M. Selection in diabetes in modern society. *Acta Med Scand* 173 459 1963
111. HARVALD B. & HAUGE, M. Hereditary factors elucidated by twin studies. U.S. Public Health Service. *Acta Med Scand* suppl 445 1965
112. HARVEY A. M. Gout. *The Principles and Practice of Medicine* 17 886, 1968
113. HAUGE, M. & HARVALD B. Heredity in gout and hyperurcemia. *Acta Med Scand* 152 247 1955
114. HAY ID R. Smoking and health: the 1972 situation. *New Engl J Med* 76 4, 1972
115. HAWK, P. H. OSER, B. L. & SUMMERSON, W. H. *Pract Physiol. Chem* 13 559 1954
116. HED R. Clinical studies in chronic alcoholism. I. Incidence of diabetes mellitus in portal cirrhosis. *Acta Med Scand* 162 189 1958
117. HED R. Clinical studies in chronic alcoholism. II. Carbohydrate metabolism in chronic alcoholism with particular reference to glucose and insulin tolerances. *Acta Med Scand* 162 195 1958
118. HED R. & NYGREN A. Alcohol-induced hypoglycemia in chronic alcoholics with liver disease. *Acta Med Scand* 183 507 1968
119. HELMBERG A. Heritability of serum lipid and lipoprotein levels. A twin study III. *Int Symp Alcoholism Berlin* Oct. 24-28 1973. In abstracts
120. HELFANT R. H. BANKA, V. S. DeVILLA, H. A. PINE, R. KADBE, V. & MEISTER, S. G. Use of bicycle ergometer and sustained handgrip exercise in the diagnosis of presence and extent of coronary heart disease. *B Heart J* 33 1321 1973
121. HELMERS, C. Short and long-term prognostic indices in acute myocardial infarction. A study of 606 patients initially treated in a coronary care unit. *Acta Med Scand* suppl. 555 1974
122. HEPTINSTALL, R. H. & PORTER, K. A. The effect of brief period of high blood pressure on cholesterol-induced atheroma in rabbits. *Br J Exp Pathol* 38 55 1957
123. HOLMBERG G. & MARTENS, S. Electroencephalographic changes in man correlated with blood alcohol concentration and some other conditions following standardized ingestion of alcohol. *Q J Stud Alcohol* 16 411, 1955
124. HEYDEN S. CASSEL, J. C. BARTEL, A. TY. ROLER, H. A., HAMES, C. G. & CORNONI, J. C. Body weight and cigarette smoking as risk factors. *Arch Intern Med* 128 915 1971
125. HIGGINS, M. W. KJELSBURG, M. & METZNER, H. Characteristics of smokers and non-smokers in Tecumseh, Michigan. I. The distribution of smoking habits in persons and families and their relationship to social characteristics. *Am J Epidemiol* 86 45 1967
126. HIGGINS, M. W. & KJELSBURG M. Characteristics of smokers and non-smokers in Tecumseh, Michigan. II. The distribution of selected physical measurements and physiologic variables and the prevalence of certain diseases in smokers and non-smokers. *Am J Epidemiol* 86 60, 1967
127. HINES, E. E. J. McILHANEY M. L. & GAGE, R. H. A study of twins with normal blood pressures and with hypertension. *Trans Assoc Am Physicians* 70 282 1957

128. HIRST A. E. HADLEY G. G. & GORE, L. The effect of chronic alcoholism and cirrhosis of the liver on atherosclerosis. *Am J Med Sci* 249 143, 1965
129. HOFVENDAHL, S. Influence of treatment in coronary care unit on prognosis in acute myocardial infarction. A controlled study in 271 cases. *Acta Med Scand suppl.* 519 1971
130. HOLLSTEDT C. Stencil för alkoholberäkning i gram absolut alkohol. Institutionen för Teoretisk Alkoholforskning, Karolinska Institutet, 1973.
131. HOLMGREN A. & STRANDELL, T. On the use of chest lead leads for recording of electrocardiogram during exercise. *Acta Med Scand* 169 57 1961
132. HOOD B. Symposium. Urnsyra, gult, jättar blodtryck och hjärt. *Läkartidn* 66 65 535 1968
133. HOWELL, W. L. & MANTON W. C. The low incidence of myocardial infarction in patients with portal cirrhosis of the liver—review of 639 cases of cirrhosis of the liver from 17731 autopsies. *Am Heart J* 60 341, 1960
134. HUSS, M. Alkoholismens kroniska eller kroniska alkoholsjukdom. Thesis, Stockholm 1949
135. IKKOS, D. & LUFT R. On the intravenous glucose tolerance test. *Acta Endocrinol* 25 312, 1957
136. ISBEL, H., FRASER, H. F. WICKER, A. BELLEVILLE, R. E. & EISENMAN, A. J. An experimental study of the etiology of rum fits and delirium tremens. *Q J Med Alcohol* 16 1 1955
137. ISSELBACHER, K. J. & GREENBERGER, N. J. Metabolic effects of alcohol on the liver. *N Engl J Med* 270 351, 1964
138. JAMES, T. N. & BEAR, E. S. Effects of ethanol and acetaldehyde on the heart. *Am Heart J* 74 234, 1967
139. JELLINEK, E. M. The disease concept of alcoholism. *Hillhouse Press* New Haven, Conn. 1960
140. JENSEN J. BLANKENHORN, D. H., CHIN, H. & STURGEON, F. & WAILE, A. G. Serum lipids and serum stic acid in human cases. *J Lipid Res* 6 193, 1965
141. JOHANSSON, B. G. & LAURELL, C. B. Disorders of serum α -lipoproteins after alcoholic intoxication. *Scand J Clin Lab Invest* 23 231, 1969
142. JOHANSSON, B. G. Kastrum hos alkoholister. *Läkartidn* 66 3715 1969
143. JONSELL, S. A method for the determination of the heart size by teleroentgenography (A heart volume index). *Acta Radiol (Stockh.)* 20:325 1959
144. JONSSON E. & NILSSON T. Alkoholkonsumtion hos monogyna och drygyna tillbringar. *Nord Nys Tid* 1 49:21, 1968.
145. JØRGENSEN F. S. & HEDEBO, S. Elektrokardiografiske undersøgelser hos kroniske alkoholkere. *Nord Med* 80 1441, 1968
146. KAIJ L. Alcoholism in twins. Thesis. From the Department of Psychiatry of Lund. 1960
147. KAKOLEWSKI, J. W. & HIRWICH, H. E. Effects of ethanol on ECG and blood pressure in the rabbit. *Q J Med Alcohol* 29 790, 1968.
148. KANNEL, W. B. LEBLAUR, E. J. DAWBER, T. R. & McNAMARA, P. M. Relation of body weight to development of coronary heart disease. The Framingham study. *Circulation* 35 734, 1967
149. KANNEL, W. B. CASTELLI, W. P. & McNAMARA, P. M. The coronary profile: 12-year follow-up in the Framingham study. *J Chron Med* 9 611 1967
150. KANNEL, W. B., CASTELLI, W. P. & McNAMARA, P. M. Cigarette smoking and risk of coronary heart disease. Epidemiologic clues to pathogenesis. The Framingham study. *Nat Genet* 1 21 1968
151. KANNEL, W. B., CASTELLI, W. P. GORDON T. & McNAMARA, P. M. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. *Am J Intern Med* 74.1, 1971
152. KANNEL, W. B. GORDON T. & SCHWARTZ, M. J. Systolic versus diastolic blood pressure and risk of coronary heart disease. The Framingham study. *Am J Cardiol* 27 335 1971
153. KARVONEN, M. J. TELIVUO L. J. & JÄRVINEN E. J. K. Sphygmomanometer cuff size and the accuracy of indirect measurement of blood pressure. *Am J Cardiol* 13 688, 1964.
154. KEEN, H. ROSE, G., PYKE, D. A. BOYNS, D. CHLOUVERAKIS, C. & MISTRY S. Blood-sugar and arterial disease. *Lancet* 2 505 1965
155. KESSLER, G. & LEIDLER, H. Fluorometric measurement of triglycerides. *Technicon Symposium* New York, 63—p 29 1965
156. KEYS, A. Epidemiological studies related to coronary heart disease. Characteristics of men aged 40—59 in seven countries. *Acta Med Scand suppl.* 460, 1966.
157. KEYS, A. Coronary heart disease in seven countries. *Circulation suppl.* 1 186, 1970.
158. KEYS, A., TAYLOR, H. L., BLACKBURN H., BROZEK, J. ANDERSSON, J. T. & SIMONSON, E. Mortality and coronary heart disease among men studied for 35 years. *Arch Intern Med* 128:201, 1971
159. KEYS, A., ARAVANIS, C., BLACKBURN H., DJORDJEVIC, B. S. DONTAS, A. S. FIDANZA, F. KARVONEN, M. J. MENOTTI, A. & TAYLOR, H. L. Lung function as risk factor

- for coronary heart disease. *Am J P M Health* 62 1306, 1972.
- 160 KEYS, A., ARAVANIS, C. BLACKBURN H., van BUCHEM, P. & P. BUZINA, R., DJORDJEVIC, B. S., FIDANZA, F. & KARVONEN M. J. et al. Coronary heart disease: overweight and obesity as risk factors. *Am Intern Med* 77 13 1972.
- 161 KING G. E. Taking the blood pressure. *JAMA* 209 1902, 1969
- 162 KINSEY D. WALTHER, R., SISE, H. S. WHITEHEAD G. & SMITHWICK, R. Incidence of hyperuricemia in 400 hypertensive patients. *Circulation* 24 972, 1961
- 163 KJELDSEN, K., ASTRUP P. & WANSTRUP J. Ultrastructural cellular changes in the rabbit aorta after moderate carbon monoxide exposure. *Alber clerus* 16 67 1972.
- 164 KLEIN, R., KLEIN B. E., CORNOLI, J. C., MAREADY J. CASSEL, J. C. & TYROLER, H. A. Serum uric acid, its relationship to coronary heart disease risk factors and cardiovascular disease, Evans county Georgia. *Arch Intern Med* 132 401 1973.
- 165 KÖLBEL, F. GREGOROVA, I. & SONKA, J. Hyperuricemia in hypertension. *Len et* 1 319, 1965
- 166 KOSOWSKY B. D. LOWN B. WHITING R. & GUINEY T. Occurrence of ventricular arrhythmias with exercise as compared to monitoring. *Circulation* 44 826, 1971
- 167 KULLER, L. & TONASCIA, S. A follow-up study of the commission on chronic disease morbidity survey in Baltimore—IV Factors influencing mortality from stroke and arteriosclerotic heart disease (1954—1967). *J Chro Di* 24 111 1971
- 168 LEFEVRE, A. F. DeCARLI L. M. & LIEBER, C. S. Effect of ethanol on cholesterol and bile acid metabolism. *J Lipid Res* 13 48, 1972
- 169 LEVINE, H. D. FLEMING, T. E. & AMER, K. E. A brisk electrocardiogram observed in chronic alcoholics. *Am Heart J* 69 140 1965
- 170 LEVINE, J. B. & ZAK, B. Automatic determination of serum total cholesterol. *Clin Chem Acta* 10 381 1964
- 171 LIEBER, C. S. JONES, D. P. LOSOWSKY M. S. & DAVIDSON C. S. Interrelation of uric acid and ethanol metabolism in man. *J Clin Invest* 41 1863 1962.
- 172 LIEBER, C. S. JONES, D. F. MENDELSON J. & DeCARLI, L. M. Fatty liver hyperlipemia and hyperuricemia produced by prolonged alcohol consumption, despite adequate dietary intake. *Trans Assoc Am Physicians* 76 289 1963.
- 173 LILJEPORS I. Coronary heart disease in male twins. Hereditary and environmental factors in concordant and discordant pairs. *Acta Med Scand suppl.* 511, 1970
- 174 LILJESTRAND G. LYSHOLM, E., NYLIN G. & ZACHRISSON, C. G. The normal heart volume in man. *Am Heart J* 17 406, 1939
- 175 LINDEN L. Prognostic aspects of myocardial infarction. *Acta Med Scand* 143 464, 1952.
- 176 LOSOWSKY M. S. JONES, D. P. DAVIDSON, C. S. & LIEBER, C. S. Studies of alcoholic hyperlipemia and its mechanism. *Am J Med* 35 794, 1963
- 177 LUNDMAN T. Smoking in relation to coronary heart disease and lung function in twins. *Acta Med Scand suppl.* 455 1966.
- 178 LUNDMAN, T. Genetic predisposition of ischemic heart disease—twin studies. The possibility of prediction, p. 186. *Scandinavian Symposium*. Nord. Bokh. Stockholm 1973
- 179 LUNDMAN T. LILJEPORS, I., CEDERLÖF R. & FRIBERG, L. The validity of the questionnaire diagnosis. *Arch Environ Health* 22 597 1971
- 180 LUNDQUIST G. Glucose tolerance in alcoholism. *Br J Addict.* 61 51 1965
- 181 MACLACHLAN, M. J. & RODMAN, G. P. Effects of food, fast and alcohol on serum uric acid and acute attacks of gout. *Am J Med* 42 38, 1967
- 182 MALMROS, H. & WIGAND G. Atherosclerosis and deficiency of essential fatty acids. *Len et* 2 749 1959
- 183 MARRACH, G. & SCHWERTZ, M. T. Effects physiologiques de l'alcool et de la caféine. cours d'ensemble chez l'homme. *Arch Sci Physiol* III 163, 1961
- 184 MARBLE, A. et al. Joslin Diabetes Mellitus, 11th Edition, Lea and Febiger Philadelphia 1971
- 185 MASON R., LIKAR, I. BIERN R. & ROSS, R. Multiple lead exercise electrocardiography. Experience in 107 normal subjects and 67 patients with angina pectoris, and comparison with coronary cinearteriography in 81 patients. *Circulation* 36 517 1967
- 186 MASTER, A. M. JAFFE, H. L. & CHESKY K. Relationship of obesity to coronary disease and hypertension. *JAMA* 153 1499 1953
- 187 MATHERS, J. A. L. OSBORNE, R. H. & De GEORGE, F. V. Studies of blood pressure, heart rate, and the electrocardiogram in adult twins. *Am Heart J* 62 634, 1961
- 188 MATHISEN H. S. LØKEN, H. BROX, O. & STENBAEK, O. The prognosis in long term treated and untreated essential hypertension. *Acta Med Scand* 183 233, 1969

- 189 MATTINGLY T W The postexercise electrocardiogram. Its value in the diagnosis and prognosis of coronary arterial disease *Am J Cardiol* 9 595 1962.
- 190 McDONOUGH, J R HAMES, C G GREENBERG B G GRIFFIN L H, J & EDWARDS, A J J Observations on serum cholesterol levels in the twin population of Evans County Georgia *Circulation* 25-1962, 1962
- 191 MENDELSON J H & MELLO N K Alcohol-induced hyperlipidemia and beta lipoproteins. *Science* 180 1372, 1973
- 192 MEYER, K. Serum cholesterol and heredity A twin study *Acta Med Scand* 172 401 1962.
- 193 MYERS, A. R., EPSTEIN, F H DODGE, H J & MIKKELSEN, W M. The relationship of serum uric acid to risk factors on coronary heart disease. *Am J Med* 45 520, 1968
- 194 MYRHED M & BERGSTROM, K. Nylga lever enzymen hos alkoholistkorsarna (villgäppar) Paper read at the Annual Meeting of the Swedish Society of Medical Sciences, Stockholm, 1973. Preprint in progress, p. 171 1973
- 195 National Heart Foundation of New Zealand Coronary heart disease: A new Zealand report Dunedin: John McIndoe Ltd 1971
- 196 NESTLE, W J & HIRSCH, E Z Mechanism of alcohol-induced hypertriglyceridemia. *J Lab Clin Med* 66 357 1965
- 197 NEWLAND H. Antagonism of the antithrombotic effect of warfarin by uric acid *Am J Med Sci* 256 44, 1968.
- 198 NIELSEN B. L. & KESSING, S. V Hepatic cirrhosis and atherosclerotic heart disease *Gastroenterology* 20 374, 1965
- 199 NILSSON, T Faktorer av betydelse för orsaken till den individuella alkoholförbrukningen — specifika sociala faktorer Institutionen för teoretisk alkoholforskning och Institutionen för hygien, Karolinska Institutet, Stockholm 1973
- 200 NOBLE, R. P. Electrophoretic separation of plasma lipoproteins in agarose gel *J Lip Res* 9 693 1968.
- 201 OPALEVA STEGANTSEVA, V A GAVRILINA, I A & SOLOV, L I. The role of alcohol in the inception of acute coronary insufficiency *Kardiologi* 12 111 1972.
- 202 OSBORN, R H ADLERSBERG, D DeGEORGE, F V & WANG, C. Serum lipids, heredity and environment. A study of adult twins. *Am J Med* 6 54, 1959
- 203 OSBORN, R H DeGEORGE, F V & MATHERS, J A. L. The variability of blood pressure: Basal and casual measurements in adult twins. *Am J Med Sci* 66 176, 1963.
- 204 OSTRANDER, L D J FRANCIS, T J HAYNER, N. S KJELLSBERG, M. B & EPSTEIN, F H The relationship of cardiovascular disease to hyperglycemia. *Ann Intern Med* 61 1188, 1965
- 205 OSTLYNGEN E. Possibilities and limitations of twin research as means of solving problems of heredity and environment. *Acta Psych* 6 111 1949
- 206 PAASIKIVI, J Long-term tolbutamide treatment after myocardial infarction. A clinical and biochemical study of 178 patients without overt diabetes. *Acta Med Scand suppl.* 307 1970.
- 207 PARTAMIAN J O & BRADLEY R. F Acute myocardial infarction in 238 cases of diabetes. Immediate mortality and five-year survival. *N Engl J Med* 273 455 1965
- 208 PARTANEN, J BRUUN, K. & MARIKKANEN, T Inheritance of drinking behavior A study on intelligence, personality and use of alcohol of adult twins. Inheritance of drinking behavior. *The Finnish Foundation for Alcohol Studies* 1966
- 209 PAUL, O Risks of mild hypertension: ten-year report. *B Heart J* 33 116, 1971
- 210 PELL, S. & D'ALONZO C. A. A five-year mortality study of alcoholics. *J Occup Med* 15 120 1973
- 211 PERMAN, E. S Effect of ethanol on oxygen uptake and on blood glucose concentration in anesthetized rabbits. *Acta Physiol. Scand* 55 189 1962
- 212 PHILLIPS, G. B Effect of alcohol on glucose tolerance. *Lancet* 2 1317 1971
- 213 PHILLIPS, G B & SAFRIT H F Alcoholic diabetes. Induction of glucose intolerance with alcohol. *JAMA* 217 1513, 1971
- 214 PRIEST R. G BINNS, J K. & KITCHIN, A. H Electrocardiogram in alcoholism and accompanying physical disease. *Br Med J* 1 1455 1966.
- 215 PRIOR, I. A M ROSE, B S, HARVEY H P B & DAVIDSON F Hyperuricaemia, gout, and diabetic abnormality in Polynesian people. *Lancet* 1 333, 1966.
- 216 PYÖRÄLÄ, K. Blood Glucose, Plasma Insulin and Coronary heart disease *Ad Cardiol.* 8 100, 1973.
- 217 RAFTERY E. B, COCKER, F & HOLLAND W W Value of electrocardiogram in population surveys. An attempt to improve interpretation *Br Heart J* 33-437 1971.
- 218 Rapport 73 Alkohol och narkotika. Centralförbundet för alkohol- och narkotikupplysning, Stockholm 1973
- 219 REMINGTON, R. & SCHORK, M. Statistics

- with applications to the biological and health sciences. New York. *Prattice-Hall* 1970
220. RIFKIND B. M. BOYLE, J. A., GALE, M. GREIG W. & BUCHANAN, W. W. Study of serum lipid levels in twins. *Cardio vas Res* 2 148, 1968.
 221. ROSALKI, S. B. Screening test for alcoholism. *Lancet* 2:843, 1973
 222. ROSALKI, S. B. & RAU E. Serum gamma-glutamyl transpeptidase activity in alcoholism. *Clin Chim Acta* 39:41 1972.
 223. ROSE, G. A. Validation of the angina questionnaire WHO Scientific Group on Comparable Methodology for the Epidemiological Study of Hypertension and Ischaemic Heart Disease. *Med.* 6, 1961
 224. ROSE, G. A. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. *Ball Wld Hlth Org* 27 645 1962.
 225. ROSE, G. Familial patterns in ischaemic heart disease. *Br J Prev Soc Med* 18 75, 1964
 226. ROSE, G. Variability of angina some implications for epidemiology. *Br J Prev Soc Med* 22 12, 1968.
 227. ROSE, G. Smoking and cardiovascular disease. *Am Heart J* 85:838, 1973
 228. ROSE, G. & BLACKBURN H. Cardiovascular survey methods. Geneva, W H O 1968.
 229. Royal College of Physicians. Smoking and diseases of the heart and blood vessels. Smoking and health. *Prattice Med and Sci P H Co Ltd* London, 1962.
 230. Royal College of Physicians. Smoking and diseases of the heart and blood vessels. Smoking and Health. Now *Prattice Med and Sci P H Co Ltd* London, 1971
 231. RUEBNER, B. H. MIYAI, K. & ABBEY H. The low incidence of myocardial infarction in hepatic cirrhosis. *Lancet* 2 1435 1961
 232. RUSSEK, H. I. & ZOHMAN, B. I. Relative significance of heredity diet and occupational stress in coronary heart disease of young adults. Based on an analysis of 100 patients between the ages of 25 and 40 years and similar group of 100 normal control subjects. *Am J Med. Sci* 255 266, 1958
 233. SCHMIDT W. & deLINT J. Causes of death of alcoholics. *Q J Stud Alcohol* 35 171 1972
 234. SCHLICKIT M. A. GOODWIN, E. W. & WINOKUR, G. Genetic investigation in alcoholism—the half-sibling approach. Finnish foundation for alcohol studies. *Int Symp* 20 163 1972
 235. SHANLEY B. C. ROBERTSON E. J. JOE BERT S. M. & NORTHCOOMBS, J. Effect of alcohol on glucose tolerance. *Lancet* 1 1332, 1972
 36. SHERLOCK, S. Diseases of the liver and the biliary system. Fourth Edition, Blackwell, Oxford and Edinburgh, 1968
 237. SIEGEL, S. Nonparametric statistics. McGraw Hill Book Company N Y Toronto, London, 1956.
 238. SIEVERS J. Myocardial infarction. *Acta Med Scand* suppl 406, 1965
 239. SIEVERS, J. BLOMQUIST G. & BJÖRCK, G. Studies on myocardial infarction in Malmö 1935 to 1951. VI Some clinical data with particular reference to diabetes, menopause and heart rupture. *Acta Med Scand* 169:95 1961
 240. SIBBORG, D. W. The status of risk factors and coronary heart disease. *J Chro Dis* 22 315 1970
 241. SIMPSON, J. A., JAMIESON G. DICKHAUS, D. W. & GROVER, R. F. Effect of size cuff bladder on accuracy of measurement of indirect blood pressure. *Am Heart J* 70:208, 1965
 242. SIRTORI C. R., AGRADI, E., MARIANI, C., CANAL, N. & PRATTOLA, L. Alcoholic hyperlipidemia. *Lancet* 2:820 1972
 243. SMYTH, C. J. Symposium on gout. Hereditary factors in gout. A review of recent literature. *Metabolism* 6 218 1957
 244. SPODICK, D. H. PIGOTT V. M. & CHURFF, H. Preclinical cardiac malfunction in chronic alcoholism. Comparison with matched normal controls and with alcoholic cardiomyopathy. *N Engl J Med* 287 677 1972
 245. STAMLER, J. Atherosclerotic coronary heart disease—etiology and pathogenesis the coronary risk factors. In: Lectures on preventive cardiology p. 107 Grune and Stratton Inc. N Y and London, 1967
 246. STAMLER, J. Epidemiology of coronary heart disease. *Med Clin North Am* 57 5 1973
 247. STARE, P. Myocardial infarction in patients with portal cirrhosis. *Nutr Res* 19 37 1961
 248. STEIN, S. W. LIEBER, C. S. LEEVY C. M., CHERRICK, G. R. & ABELMANN W. H. The effect of ethanol upon systemic and hepatic blood flow in man. *Am J Cls Nutr* 15 60 1963
 249. SUNDBY P. Alcoholism and mortality. *Universitetsforlaget* Oslo, 1967
 250. SZASZ, G. A kinetic photometric method for serum gamma-glutamyl transpeptidase. *Clin Chem* 15 124, 1969
 251. TAKUNEN, J. Anthropometric, electrocardio-

- graphic and blood pressure studies on adult male twins. *Ann Acad Sci Fenn* 1964
252. TALBOTT J H Serum urate in relation of gouty patients. *J Clin Invest* 19 643 1940.
 253. THEN BERG, H. Die Erbbiologie des Diabetes Mellitus. Vorläufiges Ergebnis der Züchtungsuntersuchungen. *Arch f Rassen- u G Biol* 32 289 1938.
 254. THEORELL, T Psychosocial factors in relation to the onset of myocardial infarction and to some metabolic variables—a pilot study Academic thesis. Karol. Inst., 1971
 255. THOMAS, C. B. Familial and epidemiologic aspects of coronary disease and hypertension. *J Chron Dis* 7 199 1958
 256. THOMAS, C. B. Familial patterns in hypertension and coronary heart disease. *Circulation* 20:25 1959
 257. THOMAS, C. B. The relationship of smoking and habits of nervous tension. In smoking behavior. Motives and incentives. Editor W. L. Dunn J. Winston and Sons Inc. Washington, p. 157 1973
 258. TIBBLIN G. High blood pressure in men aged 50. A population study of men born in 1913. *Acta Med Scand suppl* 470, 1967
 259. TIBBLIN G. Primära och sekundära riskfaktorer. *Läkertidnngen* 69 4645 1972
 260. TOFLER, O H SAKER, B. M. ROLLO K A BULVILL, M. J & STENHOUSE, N. Electrocardiogram of the social drinker in Perth, Western Australia. *Br Heart J* 31 306, 1969
 261. Twin registries in the study of chronic disease. With particular reference to the relation of smoking to cardiovascular and pulmonary disease. Report of an International Symposium in San Juan, Puerto Rico, 1—4 december 1969. *Acta Med Scand suppl* 523, 1971
 262. U. S. Public Health Service Smoking and Health. *Public Health Serv. P M* 1103 316, 1964.
 263. U. S. Public Health Service Smoking and cardiovascular diseases. *Public Health Serv. P M* 1696, 13, 1968
 264. U. S. Public Health Service The Health Consequences of Smoking. A report of the Surgeon-General Washington. U. S. Department of Health, Education and Welfare, 1971
 265. United States Public Health Service Hospitals Cooperative Study Group Morbidity and mortality mild essential hypertension. *Circ Res Suppl* 2 1972.
 266. WAHLBERG, F. The intravenous glucose tolerance test in atherosclerotic disease with special reference to obesity hypertension, diabetic heredity and cholesterol values. *Acta Med Scand* 171 1 1962
 267. WAHLBERG F. Intravenous glucose tolerance in myocardial infarction, angina pectoris and intermittent claudication. *Acta Med Scand suppl* 453, 1966
 268. WAKERLIN, G. E., MOSS, W. G. & KIELY J. P. Effect of experimental renal hypertension on experimental thoracic-cholesterol atherosclerosis in dogs. *Circ Res* 5 426, 1957
 269. WALD N HOWARD, S SMITH, P G & KJELDSEN S. Association between atherosclerotic diseases and carboxyhaemoglobin levels in tobacco smokers. *Br Med J* 1 761 1973
 270. WALKER, W. J & GREGORATUS, G. Myocardial infarction in young men. *Am J Cardiol* 19:339 1967
 271. WALLGREN, H & BARRY H. III. Actions of alcohol, part I—II Elsevier Publ. Company Amsterdam — London — N. Y. 1970
 272. WAPNICK, S & JONES, J. J. Alcohol and glucose tolerance. *Lancet* 2 180 1971.
 273. WEBB, W. R. & DEGERLI, I. U. Ethyl alcohol and the cardiovascular system. Effects on coronary blood flow. *JAMA* 191 1055 1965
 274. VEDIN, J A., WILHELMSSON C., ELMFELDT D TIBBLIN, G WILHELMSEN, L & WERKÖ L. Sudden death identification of high risk groups. *Am Heart J* 86 124, 1973.
 275. WELBORN, T A., CLUMPSTON, G N CULLEN, K. J CURNOW D H., McCALL, M. G. & STENHOUSE, N. S. The prevalence of coronary heart disease and associated factors in an Australian rural community. *Am J Epidemiol.* 89- 321, 1969
 276. VERSCHUER, O V Die Zwillingforschung im Dienste der Inneren Medizin. *Arch Dis & Geriatr Med* 64 262, 1958.
 277. Veterans administration cooperative study group on antihypertensive agents. Effects of treatment on morbidity in hypertension. Results in patients with diastolic blood pressures averaging 115 through 129 mm Hg. *JAMA* 202 1028 1967
 278. "Veterans administrations cooperative study group on antihypertensive agents. Effects of treatment on morbidity in hypertension. II. Results in patients with diastolic blood pressure averaging 90 through 114 mm Hg. *JAMA* 213 1143, 1970.
 279. Veterans administration cooperative study group on antihypertensive agents. Effects of treatment on morbidity in hypertension. III. Influence of age, diastolic pressure, and prior cardiovascular disease. Further analysis of side effects. *Circulation* 45:991, 1972.

280. VETTER, W. R., COHN L. H. & REICHGOTT M.. Hypokalemia and electrocardiographic abnormalities during acute alcohol withdrawal. *Arch Int Med* 120 336, 1967
281. WIEBE, T. LUNDQUIST A. & BELFRAGE, P. Time-course of liver fat accumulation in man after single load of ethanol. *Scand J Clin Lab I* 27 33, 1971
282. WIEHELMSEN L., WEDEL, H. & TIBBLIN G. Multivariate analysis of risk factors for coronary heart disease *Circulation* 48 950 1973.
283. WILKINSON C. F. Jr. HAND, E. A. & FLIE GELMAN M. T. Essential familial hypercholesterolemia. *Ann Intern Med* 79 671 1948.
284. VILLIGER, U. & HEYDEN S.. Neue Ergebnisse der epidemiologischen Forschung bei der ischämischen Herzerkrankung. 2. Teil I. Retrospektive epidemiologische Beobachtung: Der Alkoholkonsum des Infarktpatienten. *Z Kreislaufforsch.* 55 738, 1966.
285. WINOKUR, G. REICH, T. RIMMER, J. & PITTS, F. N. J. Alcoholism. III. Diagnosis and familial psychiatric illness in 259 alcoholic probands. *Arch Gen Psychiat* 13 104, 1970

Appendix

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Supplementum 568

Ischaemic Heart Disease in Death Discordant Twins

A Study on 205 Male and Female Pairs

By Ulf de Faire

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An increasing mortality from ischaemic heart disease (IHD) during the last decade especially in men has been reported by several authors (d Haas 1964; Oliver & Stuart-Harris 1965; Hader & Wynn 1966; Stamler 1973). However, figures from mortality statistics in Sweden show only a slight increase in men and a corresponding decrease in women (Björck & Bylin 1965; Björck et al. 1970; Vedin et al. 1971). In comparisons between countries, male IHD mortality is high in Finland and the USA while Sweden comes comparatively low down (Blomqvist & Björck 1963; Moriyama et al. 1971).

IHD is no doubt a major international problem causing early death and invalidity. The National Health Examination Survey has shown that several million persons in the USA have IHD definite or suspect (Athlete Clinical Study Group 1970). Therefore it is understandable that great efforts are now concentrated on trying to prevent or at least delay the development of IHD (primary prevention) as well as trying to prevent relapse (secondary prevention). However, preventive action presupposes knowledge of the pertinent etiological factors.

Prospective population studies have demonstrated associations between the incidence of IHD and different variables usually called risk factors. By risk factor are understood those factors which are statistically associated with IHD but which do not necessarily imply a causal relationship.

Many extensive reviews concerning risk factors have been published (Kannel et al. 1961; Björck 1963; Eys & Blackburn 1963; Epstein 1967; Simsborg 1970; Ejjar 1972; Stamler 1973). Judging from several prospective studies, the major contributory factors seem to be hypertension, hypercholesterolaemia and smoking (Athlete Clinical Study Group 1970). Their impact on risk is more pronounced when they are present in combination. Other risk factors have also been identified as for example hypertriglyceridaemia, various hyperlipoproteinaemias, overweight, sedentary living, psychosocial tensions, dietary factors, excessive alcohol consumption, hyperuricaemia and diabetes mellitus. The risk factor can be subdivided into biometric, intrinsic and environmental or extrinsic. The role of many biometric factors are probably mostly genetic but they may be susceptible to the influence of environment. Similarly

environmental factors may be linked in some measure to the genetic constitution (Bisrock 1959)

The importance of the hereditary influence in IHD has always been stressed by Paul D. Whit (1957 and 1960). The general approaches to determining the strength and mechanism of genetic factors in chronic diseases have been described among others by Penrose (1953) and Edwards (1963). The methods available for observational study of the genetic basis of IHD have usually been the study of familial aggregation and twin studies.

Familial aggregation of ischaemic heart disease

Familial aggregation of IHD has often been accepted as an indication of a substantial genetic influence on IHD. Several studies have demonstrated such aggregation (Gertler & White 1954; Thomas & Cohen 1955; Ruick & Zohmann 1958; Shanoff et al 1961; Rose 1964). However, as pointed out by McKusick and Murphy (1963), modest familial concentrations have been found for most common diseases for which they have been sought. A contributory factor to the familial clustering of IHD found in retrospective studies could be that probands are more aware of relatives with the trait in question. In a report from the Tecumseh Community Health Study, Napier et al (1972) have shown that family history reports on morbidity are rather limited. This finding also suggested that mortality data obtained exclusively from family histories should be interpreted with great caution.

Prospective studies have also disclosed familial aggregation of IHD (Slack & Evans 1966; Dentcher et al 1970; Hammond et al 1971). But according to Epstein (1964), the relative importance of familial influences in the genesis of IHD cannot be estimated quantitatively from the findings of family studies. It must be born in mind that the members of a family tend to share not only their genes but their environment.

Twin studies

Ever since the day of Galton (1822-1911), twins have been studied with regard to the genetic influence on chronic diseases. The measurable contribution of twins in epidemiologic studies of chronic diseases was reviewed at a meeting in 1965 sponsored by WHO. It was stressed that twin studies offer a useful tool in evaluating the relative importance of heredity and environment for chronic conditions such as IHD. The methodology in twin studies has been carefully

analysed by C d rlf (1966) and oth r in luding an international symposium in Pu rto Rico in 1969. Sev ral models have b n suggested for testing specific hyp these. The most common t chnique i probably the called cla sical twin method de ribed by several author (Dahlb rg 1926; von V rschu r 1958; Gedda 1961; Harvald & Hauge 1965). Here the rate of concordance for ome p ific trait i compar d b tween monozygoti (MZ) and dizygoti (DZ) twin pairs. A ignificantly high r dgree f concordance in the MZ pair speaks in favour of geneti factors b ing r possible for th diff r nce. Thi conclusion a ume h wev r that th intra pair difference for MZ and DZ twins with re pect to environmental fa tors i of ab ut th am ord r which may n t be the case. Another assumption i an unbiased selection of pair but in pra tic there i often an over r pr entation of pairs with th trait und r inve tigation.

A imilar approach used by Kaij (1960) and Ced rlf et al (1970) i to compare th b rved and xpected rate of coin id nce. The ob served rat i d find a th ratio between th numb r of pairs where both twins hav th trait and th total number of pair.

b rved. Th xpected rate i calculated from th prevalen in the twin sample. Th gen tic dependence of c ntinuous variable and th influ n on them of environment are b t studied by varian e analysi (O born & d George 1959; Takkenen 1964). A le marked intra pair differ n in MZ pairs compared to DZ pairs indicates that the variabl in qu tion i more dep nd nt on g n tic than on environmental factor.

Th co twin control method worked out by G ll (1942) i e p ially suitable for tudyng th influen of sp cifi environmental fa tors n h alth. It has th advantage of keeping the genetic fa t r und r ntrol. A typical xample of thi approach i th tudy of smoking di ordant twins with re pect t IHD and lung fun tion (Lundman 1966).

I haemic heart disease in twins

Sev ral case rep rt hav been published con rning concordance in MZ twin pair (Parade & Lehmann 1938; Froment t al 1945; B rna on et al 1957; Benedi t 1958; Gikni et al 1965; Dougla 1966; Sidd et al 1966) but only a f w concerning di ordant pair (Sul r & Koller 1961; Le et al 1963). Although individual case reports on twin c n rdan for IHD obvious ly cannot prove th pre nce of h redity in IHD th y are suffici ntly suggestive to rai a su p i on

of its presence Kahler & Weber (1940) studied 17 twin pairs in which at least one of the twins suffered from IHD. Three of the four MZ pairs were concordant with respect to IHD but only two of the DZ pairs. Reviewing several small twin materials von Verschuer (1958) reported a combined IHD concordance of 19 % in the MZ pairs as compared to 8.5 % in the DZ pairs.

Only two representative unselected twin samples, the Danish and the Swedish Twin Registries, are suitable for epidemiological studies.

The Danish Twin Registry was founded in 1954 and has been developed ever since (Harvald & Hauge 1968). It contains information on medical and social conditions on about 8 000 unselected pairs of twins born in the period 1870 to 1910 where both partners had survived the age of five. By January 1 1968 coronary occlusion had occurred in 352 twins. The rate of concordance has been calculated by the twin proband method (Allen et al 1967), i.e. concordant pairs are counted twice as both partners are considered probands. The concordance rate in the male MZ pair 39 % differed significantly from that of 26 % in the male DZ pair. In the female pairs the difference was much greater 44 and 14 % respectively giving significance at a higher level. The risk of a co-twin dying from coronary occlusion in the first 10 years after the proband's death was also calculated. For co-twin in MZ and DZ male pair the risk curves were very similar but for the female pairs they deviated considerably right from the start. The risk of a female MZ co-twin dying from

coronary occlusion was over 40 % in the first 10 years after the proband's death as compared to about 4 % for a female DZ twin. The corresponding risks for the male MZ and DZ pairs were about 15 %. It was concluded that the occurrence of fatal coronary occlusion seemed to be genetically determined to a very limited extent in males and to a much larger extent in females.

In a questionnaire study on the Swedish Twin Registry Cederlöf et al (1967) evaluated the prevalence of angina pectoris using the questionnaires worked out by Rose (1962). The investigation comprised 2 255 MZ and 3 672 DZ twin pairs 40 to 80 years of age with concordant smoking habit. The results showed a significantly higher degree of coincidence of angina pectoris in MZ compared to DZ pair for male as well as females in the age group 60-80 years but for the younger age group 40-60 years this was true only for the females. Validation of the mailed questionnaire concerning angina pectoris (Lundman et al 1971) indicated that it is very useful for screening cases with IHD but one has to be aware of a tendency to give false positives.

In a study of 196 smoking discordant male and female twins from the Swedish Twin Registry Lundman (1966) evaluated the occurrence of segmental ST depression of at least 0.5 mm at post-exercise electrocardiography and found that the observed incidence rate was significantly higher than the expected rate in both MZ and DZ pairs. It was suggested that these segmental changes could be due to multiple dominant genes. When the clinical picture of IHD was included in the judgement of IHD the difference within the MZ pair was more pronounced but not within the DZ pairs which was considered to indicate a substantial genetic component in IHD. However, female and male twin pairs were pooled in this analysis, obstructing further conclusion. The occurrence of IHD did not differ between smoking and non-smoking twin pairs.

Liljeferg (1970) studied 91 male twin pairs, all from the Swedish Twin Registry, aged 42-67 years, who were concordant or discordant with respect to IHD. The concordance rate was calculated for different manifestations of IHD: Per myocardial infarction, concordance was 50% in one MZ pair and 0% in DZ pairs. When the concept of IHD was extended to include angina pectoris and/or pathological electrocardiographic findings (Q_2 at rest according to the Minnesota code and/or ST segmental depressions of at least 0.5 mm during exercise) to indicate the probable presence of IHD, 48.5% of the MZ pairs were concordant as compared to 28.5% of the DZ pairs. The difference was, however, not statistically significant.

Although different manifestations of IHD have been measured, the results of these few twin studies do suggest some general comments. The difference found in concordance rates between MZ and DZ pairs were smaller in males than females (cf. Harvald & Hauge and Cederlöf et al). These findings could indicate that environmental factors are strong enough to outweigh or mask the genetic influence in men but not to the same extent in women.

Background and objectives of the present study

The establishment of the Swedish Twin Registry provided a useful tool for epidemiological studies. It was set up primarily to study factors of etiologic importance for diseases of the respiratory and cardiovascular systems. The Registry is administered by the Department of Environmental Hygiene, Karolinska Institute, and a research program is carried out in collaboration with the Department of Medicine at the Serafimer Hospital. One line of this program is a continuous mortality follow-up, and the other comprises clinical studies on sub-sample

of the Twin Registry At the international twin symposium in Puerto Rico in 1969 it was pointed out that no generally accepted methodology in the design of twin studies exists and there are many complex possibilities. Studies of mortality were considered of great importance and as valuable extensions of clinical examinations of the partners of deceased twins were suggested with a view to detecting any difference in disease prevalence between surviving MZ and DZ co-twins. No such studies have yet been carried out.

From January 1971 information on deceased twins has been available every month thereby permitting clinical examination of the surviving co-twin reasonably soon after the death of the partner. This provided one of the prerequisites for the present study. By using a sample of unselected death discordant twin with surviving co-twins could be examined in an unbiased way with respect to various manifestations of IHD and risk factors. If an important genetic influence on IHD exists it is plausible that IHD silent or manifest will appear significantly more often in the most genetically predisposed twins i.e. MZ co-twin whose partner have died from IHD as compared to DZ co-twins whose partner have died from IHD. The comparison should also be extended to the co-twins whose partner have died from other causes than IHD thus permitting a comparison between different genetically predisposed surviving co-twins. The objective of the study can be summarized as follows:

- (1) Evaluate the genetic influence in IHD: is there an association between the occurrence of IHD in the surviving co-twin and the cause of death of the partner (IHD or not IHD)?
- (2) Is there an association between the risk factor profile in the surviving co-twin and the cause of death of the partner (IHD or not IHD)?
- (3) Are there environmental differences in the death discordant pairs as elucidated from earlier questionnaires?

Furthermore this study provides a basis for a continuous follow-up of mortality of the co-twins examined and thus makes it possible to assess the predictive value of the measured risk factors as well as the hereditary influence.

I MATERIAL

The material of discordant twins for the present investigation derive from the Swedish Twin Registry and the study constitutes part of the research program for the Registry (Fig. 1)

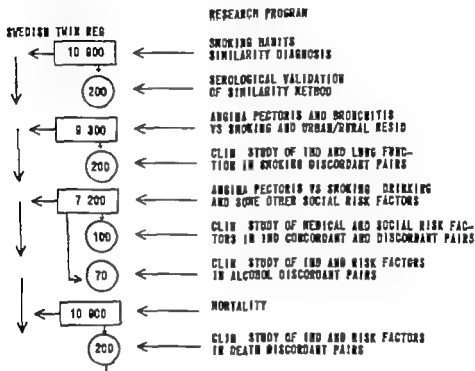


Fig. 1 Research program for the Swedish Twin Registry

The Swedish Twin Registry

The Swedish Twin Registry was set up in the years 1959 to 1961 at the Department of Environmental Hygiene, Karolinska Institute and the former National Institute of Public Health. It contains about 10 000 twin pairs and covers about 95 % of all Swedish same sex twins who were born in the country between 1886 and 1955 and with both members living when the Registry was formed. The compilation procedure and the demographic structure of the twin series have been described in detail by Cederlöf (1966 a). The Registry has been used for several questionnaire studies, mainly dealing with respiratory symptoms and angina pectoris, with special reference to smoking (Cederlöf et al.

1966 b; Cederlöf et al 1966 c; Cederlöf 1966 d; Cederlöf et al 1967 a; Cederlöf et al 1967 b) and two clinical studies (Lundman 1966; Liljefors 1970)

Zygosity diagnosis

The zygosity of the twins in the Registry has been determined with the aid of similarity questions contained in a questionnaire mailed to each one of them (Cederlöf 1966 a). One of these items proved to be of high reliability namely the very simple question whether the twins as children were as alike as two people in a pod or of family likeness only. The questions were validated by Cederlöf et al (1961) on 200 randomly selected pairs by comparing the questionnaire diagnosis with blood-group serology in respect of five independent systems namely A₁A₂BO MN Rh Hp and Gm. The MZ and the DZ diagnosis arrived at from the questionnaire agreed with the serological diagnosis in 99 % and 91 % respectively. About 4 % of the twins gave conflicting answers concerning similarity and have therefore been classified as of unknown zygosity (XZ). Excluding the XZ group the MZ twins make up 35.6 % of the men and 34.1 % of the women in the Registry. The zygosity diagnosis in the present study are exclusively based on the diagnosis earlier made in the Twin Registry.

Mortality evaluation

The mortality among the twins is tabulated as follows. The total twin registry is matched regularly against a total death registry for Sweden at the Central Bureau of Statistics. Since 1971 this has been done once a month (Boland 1973). The procedure provides access to the death certificate and the name of the physician who signed it. The certificate in turn indicates whether or not the deceased twin had been treated in a hospital. Hospital record autopsy records information from general practitioner and other pertinent information are collected. The cause of death is then established from all these records. The final evaluation has been made together with two other doctors (Dr L. Friberg and T. Lundman) and the cause of death has been classified according to the 1965 revision of International Statistical Classification (ISC 1969) of Diseases, Injuries and Cause of Death which has been used since 1969 in this country. Further detail of the mortality evaluation will be found in two reports on the mortality follow up in smoking discordant twins (Friberg et al 1970 and 1973).

Criteria for selection of the present material

During the period January 1st 1971 to March 15th 1973 262 male and female twin pairs below the age of 70 became death discordant i.e. one of the members in an unbroken pair died during the period mentioned above. About two or three months after death discordance had occurred the surviving co-twins were invited to a thorough health check up at Srafimer Hospital Stockholm. The selection procedure is visualized in Fig. 2.

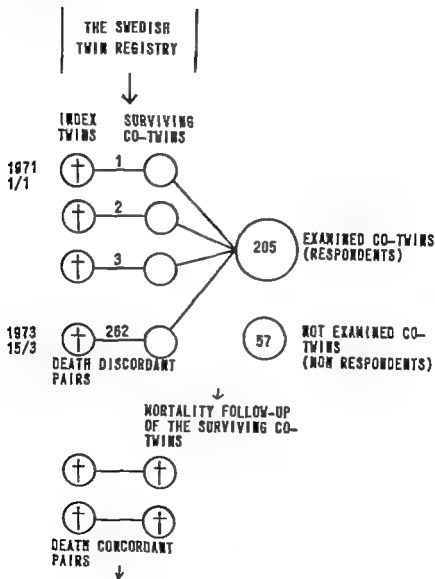


Fig. 2. Selection of the present material

Table 1 Causes of death of the index-twins with a breakdown by sex

	Males		Females		Both sexes	
	No	%	No	%	No	%
All causes	141	100.0	121	100.0	262	100.0
IHD total	48	34.0	21	17.4	69	26.3
Myocardial infarction	34	24.1	10	8.3	44	16.8
Other IHD			2	1.7	2	0.8
Sudden death	14	9.9	9	7.4	23	8.8
Not IHD total	93	66.0	100	82.6	193	73.7
CVD	10	7.1	10	8.3	20	7.6
Malignant tumor	34	24.1	53	43.8	87	33.2
Uræmia	1	0.7	2	1.7	3	1.1
Diabetes mellitus	2	1.4	2	1.7	4	1.5
Suicides	8	5.7	4	3.3	12	4.6
Accident	8	5.7	8	6.6	16	6.1
Other a/s	30	21.3	41	17.4	71	19.5

The deceased twins

The deceased twins constitute the index twins in the death discordant pairs. A breakdown by cause of death determined earlier described is given in Table 1 using the following group (ISC numbers in parenthesis): IHD (410.00-414.99-795.99) cerebrovascular disease (CVD) (430.00-438.99) malignant tumor (140.01-209.99) uræmia (792.99) diabetes mellitus (250.00-250.09) suicides (E 950.9-E 959.9) accident (800.00-999.99-E 807.0-E 949.9-E 960.9-E 999.9) and other causes (conditions not specified here but corresponding to the remaining ISC numbers). IHD here includes myocardial infarction (410.00-410.99) sudden death (795.99) and other IHD. The last heading contains only two female cases both classified as 412.09 (arteriosclerosis cordis MUD -um hypertonia). Those death discordant pairs whose index twins have died from IHD are in the following referred to as IHD death discordant. Correspondingly the pairs whose index-twins died from other causes than IHD are referred to as not IHD death discordant.

Hospital records or similar sources of information were available in 224 out of the 262 deaths (85.5%). For the remaining 38 deaths (14.5%) only death certificate were available but additional information was sought in the case from relatives and/or the surviving co-twin. An autopsy had been performed in 149 of the deceased twins or 56.9%. The cases referred to autopsy included 35 forensic post mortems (13.4%).

The IHD case included 23 cases of sudden death of which 16 (69.6 %) occurred within two hours after the onset of acute symptoms. No exact information was available about this interval in the other 7 cases.

From the collected hospital records, autopsy protocol, etc., an assessment was made of whether the deceased twin had a preexisting history of IHD and/or autopsy findings indicating IHD. A preexisting history was considered most probable if one of the following two diagnoses had been present:

- (1) Myocardial infarction confirmed at hospital or evidence of old infarction in the resting ECG
- (2) Angina pectoris on effort

A autopsy finding indicating IHD were regarded the existence of fresh or old infarction of the myocardium and/or marked arteriosclerotic changes (uncuttable arteries and/or obstructions) of the coronary arteries. Thus, of the 23 cases of sudden death, 16 (69.6 %) had either a preexisting history of IHD or a positive autopsy. The corresponding figure for the 193 cases not assigned to the IHD group was 7 (3.6 %).

The surviving co-twin

The first twins were examined in June 1971 and the last in the beginning of August 1973. At the time of the check-up the investigator (the author in all cases) was aware neither of the index-twin's cause of death nor of the zygosity.

Response rate co-twins

The response rate and cause of non-response are given in Table 2. Of 262 twins invited, 205 were examined, which gives a response rate of 78.2 %. Among those examined, 14 were unable to come to Stockholm but were willing to attend a hospital nearer their home. Of these, 3 men and 3 women were examined at Sahlgrenska Hospital in Gothenburg; two of them came from places in the County of Bohuslän north of Gothenburg, the rest lived in Gothenburg. Another two men were examined at the Central County Hospital of Uddevalla and two women at the Central County Hospital of Lidköping. One woman and one man were examined at the County Hospital of Billnäs, one man at the Central County Hospital of Karlstad and another man at the County Hospital of Finspång.

Tabl 2 Reponse rates and causes of non-response by sex and age

	MX		MX		IX		IX		Tot	
	No	%	No	%	No	%	No	%	No	%
Males:										
Total in sample	41	100.0	94	100.0	6	100.0	141	100.0		
Total examined	35	85.4	67	71.3	6	100.0	108	76.6		
Examined in Stockholm	34	82.9	61	64.9	5	83.3	100	70.9		
Examined in the province	1	2.4	6	6.4	1	16.7	8	5.7		
Not examined: total	6	14.6	27	28.7	0	0.0	33	23.4		
No contact	1	2.9	2	2.1	0	0.0	3	2.1		
Illness	2	4.9	7	7.4	0	0.0	9	6.4		
Refusal owing to work	1	2.4	7	7.4	0	0.0	8	5.7		
Refusal for other reasons	2	4.9	8	8.5	0	0.0	10	7.1		
Data losses	0	0.0	3	3.2	0	0.0	3	2.1		
Females:										
Total in sample	47	100.0	72	100.0	2	100.0	121	100.0		
Total examined	38	80.9	57	79.2	2	100.0	97	80.2		
Examined in Stockholm	35	74.5	54	75.0	2	100.0	91	75.2		
Examined in the province	3	6.4	3	4.2	0	0.0	6	5.0		
Not examined: total	9	19.1	15	20.8	0	0.0	24	19.8		
No contact	2	4.3	0	0.0	0	0.0	2	1.7		
Illness	3	6.4	2	2.8	0	0.0	5	4.1		
Refusal owing to work	1	2.1	1	1.4	0	0.0	3	2.5		
Refusal for other reasons	2	4.3	10	13.9	0	0.0	12	9.9		
Data losses	1	2.1	1	1.4	0	0.0	2	1.7		
Both sexes:										
Total in sample	88	100.0	166	100.0	8	100.0	262	100.0		
Total examined	73	83.0	124	74.7	8	100.0	205	78.2		
Examined in Stockholm	69	78.4	115	69.3	7	87.5	191	72.9		
Examined in the province	4	4.5	9	5.4	1	12.5	14	5.3		
Not examined: total	15	17.0	42	25.3	0	0.0	57	21.8		
No contact	3	3.4	2	1.2	0	0.0	5	1.9		
Illness	5	5.7	9	5.4	0	0.0	14	5.3		
Refusals owing to work	2	2.3	9	5.4	0	0.0	11	4.2		
Refusal for other reasons	4	4.5	18	10.8	0	0.0	22	8.4		
Data losses	1	1.1	4	2.4	0	0.0	5	1.9		

Table 4 Distribution of mean age (y ars) of re pondent on twins (by sex and sygosity) according to cause of death of index twin

	Mal				F males				Both area			
	MZ		DX		MZ		DX		MZ		DX	
	M	(SE)	M	(SE)	M	(SE)	M	(SE)	M	(SE)	M	(SE)
	Tot		Tot		Tot		Tot		Tot		Tot	
All au	61 1 (1 1)	60 4 (0 8)	58 0 (3 2)	60 3 (0 6)	59 7 (1 1)	61 3 (0 8)	60 3 (1 3)	60 8 (0 6)	60 4 (0 8)	60 9 (0 3)	58 6 (2 4)	60 7 (0 4)
IID t al	57 7 (1 8)	63 0 (0 8)	57 2 (3 8)	60 9 (0 9)	63 3 (2 2)	64 9 (1 8)	0 (1 4)	64 1 (1 4)	60 2 (1 5)	63 3 (0 8)	57 2 (3 8)	61 9 (0 8)
Myo ardial infarction	59 0 (1 9)	63 7 (1 0)	59 3 (4 0)	61 9 (1 0)	57 7 (4 2)	62 6 (2 9)	0 (2 4)	60 8 (2 4)	58 6 (1 7)	63 3 (0 9)	59 3 (4 0)	61 7 (0 9)
Other IID	0	0	0	0	65 0 (0 0)	69 0 (0 0)	0 (2 0)	67 0 (2 0)	65 0 (0 0)	69 0 (0 0)	0 (2 0)	67 0 (2 0)
Sudden death	52 3 (3 5)	61 0 (1 3)	48 0 (0 0)	58 0 (1 9)	67 0 (1 2)	67 3 (0 9)	0 (0 7)	67 1 (0 7)	62 2 (3 3)	62 9 (1 3)	48 0 (0 0)	61 8 (1 6)
Not IID total	6 5 (1 3)	59 0 (1 1)	62 0 (0 0)	60 3 (0 8)	58 8 (1 2)	60 9 (0 8)	60 3 (1 5)	60 1 (0 7)	60 3 (0 9)	60 0 (0 7)	61 0 (1 0)	60 2 (0 3)

The distribution of the places of residence of the respondent co-twins is shown in Fig 3. The places of residence are scattered over most of Sweden and seem to correspond fairly well with the population density.

In Table 3 the examined co-twins are distributed according to the cause of death of the index-twin. There were 57 (27.8 %) whose partners had died from IHD. 40 of the 108 male pairs (37.0 %) and 17 of the 97 female pairs (17.5 %). Sudden death caused 25 % of the IHD deaths among the male and 41.2 % among the female. It is also worth noting that of the 8 pairs of unknown zygosity (XZ) as many as 5 were male IHD death discordant.



Fig 3 Distribution of the places of residence of the respondent twins. The figure in the two circles indicates the number of co-twins living in Stockholm and Gothenburg.

The distribution of the respondent co-twins by 5-year age groups is shown in Fig 4 and Table 4 gives the means for age by the cause of death of the index-twin

The mean age at the time of death discordance for all the respondent co-twins was 60.7 years. Among the IHD death discordant pair the mean age of the male MZ pair 57.7 years was on average 5.3 years lower than that of the corresponding DZ pair. The male MZ twins whose index twins had died from causes other than IHD had a mean age of 62.5 years compared with 59.6 years for the corresponding DZ pairs. The mean ages of the male XZ pairs in the different death discordant group are about the same as for the male MZ pairs. Among the female IHD death discordant pairs the mean ages for both the XZ pairs and the DZ pair 63.3 years and 64.9 years respectively are 4.5 and 4.0 years higher than for the MZ and DZ pairs whose index twins died from other causes than IHD.

The surviving co-twins were examined on average about 5 months after the death of the index twin (Table 5) the interval ranging between two and 12 months.

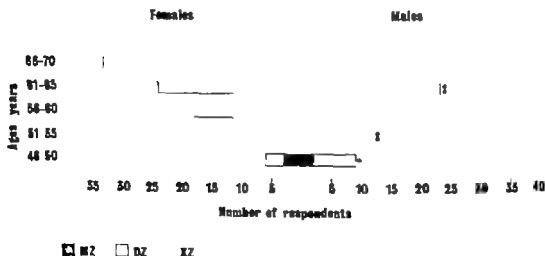


Fig 4 Distribution of respondent co-twin by 5 year age group

Table 5 Distribution of interval (mean and range in months) between death of index-twin and examination of co-twin according to cause of death of index twin

Cause of death of the index twin		MZ		DZ		XZ		Total	
		M Range		M Range		M Range		M Range	
Male	IHD	n=10	4 0-6 0	n=25	5 5-12 0	n=5	4 0-6 0	n=40	5 5-12 0
	not IHD	n=25	3 5 0-12 0	n=42	2 5 0-11 0	n=1	9 0	n=68	5 5 0-12 0
Female	IHD	n=8	3 5 0-9 0	n=9	3 5 0-10 0	n=0		n=17	3 5 0-10 0
	not IHD	n=30	2 4 0-8 0	n=48	2 5 0-11 0	n=2	5 5 0-5 5	n=80	2 5 0-11 0
Both sexes	IHD	n=18	3 5 0-9 0	n=34	2 5 5-12 0	n=5	3 4 0-6 0	n=57	2 5 5-12 0
	not IHD	n=55	2 5 0-12 0	n=90	2 5 0-11 0	n=3	5 6 0-9 0	n=148	2 5 0-12 0

Non-repondent co-twins

There were 57 twins who did not participate in the study (Table 2). Of these 5 could not be traced in spite of attempt by letter and telephone. Another 14 were unable to participate owing to illness. Two of them both were patients at mental hospital while two other men were patient at long-term clinic. One of the latter (MZ) died however in Marcus Alzheimer only 7 months after the index-twin. The cause of death attributed to the index twin was cerebrovascular disease. One female and one male twin were unable to participate because of invalidity after cerebral haemorrhage; the male twin (DZ) died from a myocardial infarction about 8 months after the death of the index twin which had been caused by cancer of the stomach. Another male twin (DZ) could not attend the examination because of general fatigue and weakness. He died later from a bronchopneumonia about 16 months after the index twin for whom the cause was cancer of the prostate gland. An unspecified neurological disorder made participation impossible for another male twin. Two twins were tied to wheel chair on women because of invalidity after poliomyelitis and one man because of a marked general rheumatoid arthritis. One

female and one male twin had recently been operated for gallbladder disease and cerebral malignant tumor respectively. Finally one woman was convalescent after a fracture of the femur and another was unable to participate because of general fatigue; she also suffered from hypertension.

Lack of time on account of work was the reason given by 11 twins (8 male and 3 female) for refusing to participate. They were all offered an examination at their home hospital. Among the other refusals were 6 twins who declared a negative attitude towards health checks in general. The remaining 16 twins in this group supplied no definite reason for not participating.

As already mentioned, the total Twin Registry is matched regularly with a total death registry for Sweden; since 1971 this is done every month. The procedure involves a slight risk of incorrect matching. Because of this, the Twin Registry has been matched twice so far with the total registry of living people in the country. The first time was in January 1971 and the second in September 1973. At the last check, which covered the time of the present investigation, it was found that 5 of the twins had been incorrectly matched. They are listed in the table as data losses.

A breakdown of the non-respondent co-twins by the cause of death of the index twins is given in Table 6. There were 12 non-responders (21.1%) whose partners had died from IHD, which is a somewhat lower proportion compared to the respondent group; this is especially true for the male. It will also be seen that there are no MZ pairs among the non-respondents. Furthermore, the percentage of MZ (the MZ omitted) among the males is less than for the respondents, 18.2 and 34.3 respectively. Among the females there are no substantial differences of this kind.

Figure 5 shows the distribution of the non-responders by 5-year age group, and Table 7 the means for age by the cause of death of the index-twin.

The mean age of the male IHD death discordant pair (61.3 years) is only 0.4 years higher than the mean for the corresponding group of respondent males. However, the non-responding male co-twins (only DZ pair) whose index twin died from myocardial infarction are on average 7.4 years younger than the corresponding male DZ twins among the responders, 56.3 years and 63.7 years respectively. The opposite age trend is seen in the non-responding male co-twin whose index twin died from sudden death (MZ + DZ pair); the mean age (66.3 years) being on average 8.3 years higher than that of the corresponding respondents.

Tabl 6 Distribution of non independent co-twin (by sex and zygosity) according to cause of death of index twin

	NUMBER OF NON RESPONDENT CO-TWINS										Both sexes	
	Male					Females						
	MZ	DZ	XX	Tot	MZ	DZ	XX	Tot	MZ	DZ	XX	Tot
All su	6	27	0	33	9	13	0	24	15	42	0	57
IMD total	1	7	0	8	2	2	0	4	3	9	0	12
Myocardial infarction	0	4	0	4	1	1	0	2	1	5	0	6
Oth = IMD	0	0	0	0	0	0	0	0	0	0	0	0
Sudd n death	1	3	0	4	1	1	0	2	2	4	0	6
Not IMD total	5	20	0	25	7	13	0	20	12	33	0	45
CVD	2	2	0	4	1	2	0	3	3	4	0	7
Malignant tumors	2	5	0	7	3	8	0	11	5	13	0	18
Ura mia	0	0	0	0	0	0	0	0	0	0	0	0
Diabet s mellitus	0	1	0	1	0	1	0	1	0	2	0	2
Suicid	0	3	0	3	1	0	0	1	1	3	0	4
Acid nt	0	1	0	1	1	1	0	2	1	2	0	3
Oth r caus	1	8	0	9	1	1	0	2	2	9	0	11

Table 7 Distribution of mean age (year) of nonrespondent co-twin (by sex and zygosity) according to number of deaths of index twin

	1st			2nd			3rd			4th			5th			6th			7th			8th			9th			10th			11th			12th			13th			14th			15th			16th			17th			18th			19th			20th			21st			22nd			23rd			24th			25th			26th			27th			28th			29th			30th			31st			32nd			33rd			34th			35th			36th			37th			38th			39th			40th			41st			42nd			43rd			44th			45th			46th			47th			48th			49th			50th			51st			52nd			53rd			54th			55th			56th			57th			58th			59th			60th			61st			62nd			63rd			64th			65th			66th			67th			68th			69th			70th			71st			72nd			73rd			74th			75th			76th			77th			78th			79th			80th			81st			82nd			83rd			84th			85th			86th			87th			88th			89th			90th			91st			92nd			93rd			94th			95th			96th			97th			98th			99th			100th			101st			102nd			103rd			104th			105th			106th			107th			108th			109th			110th			111st			112nd			113rd			114th			115th			116th			117th			118th			119th			120th			121st			122nd			123rd			124th			125th			126th			127th			128th			129th			130th			131st			132nd			133rd			134th			135th			136th			137th			138th			139th			140th			141st			142nd			143rd			144th			145th			146th			147th			148th			149th			150th			151st			152nd			153rd			154th			155th			156th			157th			158th			159th			160th			161st			162nd			163rd			164th			165th			166th			167th			168th			169th			170th			171st			172nd			173rd			174th			175th			176th			177th			178th			179th			180th			181st			182nd			183rd			184th			185th			186th			187th			188th			189th			190th			191st			192nd			193rd			194th			195th			196th			197th			198th			199th			200th			201st			202nd			203rd			204th			205th			206th			207th			208th			209th			210th			211st			212nd			213rd			214th			215th			216th			217th			218th			219th			220th			221st			222nd			223rd			224th			225th			226th			227th			228th			229th			230th			231st			232nd			233rd			234th			235th			236th			237th			238th			239th			240th			241st			242nd			243rd			244th			245th			246th			247th			248th			249th			250th			251st			252nd			253rd			254th			255th			256th			257th			258th			259th			260th			261st			262nd			263rd			264th			265th			266th			267th			268th			269th			270th			271st			272nd			273rd			274th			275th			276th			277th			278th			279th			280th			281st			282nd			283rd			284th			285th			286th			287th			288th			289th			290th			291st			292nd			293rd			294th			295th			296th			297th			298th			299th			300th			301st			302nd			303rd			304th			305th			306th			307th			308th			309th			310th			311st			312nd			313rd			314th			315th			316th			317th			318th			319th			320th			321st			322nd			323rd			324th			325th			326th			327th			328th			329th			330th			331st			332nd			333rd			334th			335th			336th			337th			338th			339th			340th			341st			342nd			343rd			344th			345th			346th			347th			348th			349th			350th			351st			352nd			353rd			354th			355th			356th			357th			358th			359th			360th			361st			362nd			363rd			364th			365th			366th			367th			368th			369th			370th			371st			372nd			373rd			374th			375th			376th			377th			378th			379th			380th			381st			382nd			383rd			384th			385th			386th			387th			388th			389th			390th			391st			392nd			393rd			394th			395th			396th			397th			398th			399th			400th			401st			402nd			403rd			404th			405th			406th			407th			408th			409th			410th			411st			412nd			413rd			414th			415th			416th			417th			418th			419th			420th			421st			422nd			423rd			424th			425th			426th			427th			428th			429th			430th			431st			432nd			433rd			434th			435th			436th			437th			438th			439th			440th			441st			442nd			443rd			444th			445th			446th			447th			448th			449th			450th			451st			452nd			453rd			454th			455th			456th			457th			458th			459th			460th			461st			462nd			463rd			464th			465th			466th			467th			468th			469th			470th			471st			472nd			473rd			474th			475th			476th			477th			478th			479th			480th			481st			482nd			483rd			484th			485th			486th			487th			488th			489th			490th			491st			492nd			493rd			494th			495th			496th			497th			498th			499th			500th			501st			502nd			503rd			504th			505th			506th			507th			508th			509th			510th			511st			512nd			513rd			514th			515th			516th			517th			518th			519th			520th			521st			522nd			523rd			524th			525th			526th			527th			528th			529th			530th			531st			532nd			533rd			534th			535th			536th			537th			538th			539th			540th			541st			542nd			543rd			544th			545th			546th			547th			548th			549th			550th			551st			552nd			553rd			554th			555th			556th			557th			558th			559th			560th			561st			562nd			563rd			564th			565th			566th			567th			568th			569th			570th			571st			572nd			573rd			574th			575th			576th			577th			578th			579th			580th			581st			582nd			583rd			584th			585th			586th			587th			588th			589th			590th			591st			592nd			593rd			594th			595th			596th			597th			598th			599th			600th			601st			602nd			603rd			604th			605th			606th			607th			608th			609th			610th			611st			612nd			613rd			614th			615th			616th			617th			618th			619th			620th			621st			622nd			623rd			624th			625th			626th			627th			628th			629th			630th			631st			632nd			633rd			634th			635th			636th			637th			638th			639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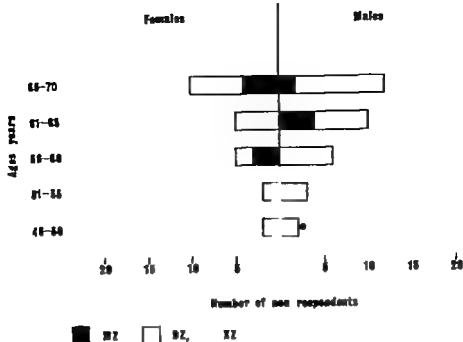


Fig 5 Distribution of non respondent by 5-year age group

Comment

One of our basic measures of the reliability of mortality statistics is probably a high autopsy rate (Britton 1974). In 1968 the autopsy rate in Sweden was about 45 % of all deaths while for the age span 16-4 years it amounted to about 50 % (Vedin et al 1971) which corresponds fairly well with the figure of about 57 % in the present material. For a correct mortality evaluation however an autopsy rate of 57 % is rather low. This is unfortunately a problem for all kind of mortality follow up which include people living outside the university city in Sweden.

The death of the index twin has been classified in 23 cases as sudden and unexpected and has been included in the IHD group. The definition of sudden death varies considerably. An international committee has agreed upon a definition to facilitate international comparisons: sudden death being defined as a death occurring immediately or within an estimated period of 24 hours after the onset of acute objective and subjective symptoms (Paul & Schatz 1971). Other time criteria are also commonly used: e.g. one hour (Chiang et al 1969 and 1970; Gordon & Kannell 1971) or two hours (Adl-son L 1961; Kuller et al 1966). In a study of 967 medically unattended deaths from IHD occurring

in Stockholm during one year (Wikland 1971) information concerning the time relationship between the onset of symptoms of the fatal attack and death was obtained in 62 %. Of these 61 % of the male and 48 % of the female case died within 15 minute of onset of symptoms. The definitions of sudden death have been carefully analysed in an editorial in Circulation (Björck & Wikland 1972). It was pointed out that the accuracy of a statement of sudden death usually depends on the observer and from that point of view it is important to note whether the preceding attack was witnessed or not (by any observer). It was furthermore concluded that for unwitnessed deaths it is difficult to know how sudden death occurred but a comparable proportion of these cases can usually be estimated to have occurred within the same time intervals as the witnessed on.

About 70 % of the sudden deaths in the present investigation occurred within two hours and were witnessed. 30 % were unwitnessed and occurred without exact information of time but probably within 24 hour. The persons who died suddenly in hospital with signs of acute myocardial damage on the ECG or at autopsy have been classified not as sudden death but as myocardial infarction. Nor were those persons included among the sudden deaths who died suddenly but with strong indications of other probable explanations for the cause of death. It is well known that especially with the 24 hour interval other causes than IHD may have been responsible for death as for instance major sudden cerebrovascular hemorrhage, pulmonary embolism, asthma bronchiale and rheumatic heart diseases especially aortic stenosis (Kuller 1966; Möhring 1969; Wikland 1971; Vedin et al 1973).

Of the 23 index twins classified as sudden deaths 16 (69.6 %) had either a pre-existing history of IHD or autopsy finding indicating the presence of IHD. Of these 16 11 were among the 16 (68.7 %) who died within two hours and 5 were among the 7 (71.4 %) who died within 24 hours. In the 7 of the 23 (30.4 %) without earlier evidence of IHD it seemed reasonable to suppose however that the sudden death was most probably the initial clinical manifestation of previously unknown IHD. This is in accordance with earlier reports which point to incidences of about 20-25 % (Kuller et al 1966; Kuller 1966).

One of the problems in epidemiological studies is the lack of complete participation. This difficulty is of course more pronounced if the population under study is drawn from the whole of Sweden instead of a more restricted area. The present response rate of 78.2 % can be compared with a rate of 79.4 % in a co-twin control study of 196 smoking discordant twins by Lundman (1966) and 87.5 % responses in

a study of IHD in 91 twin pair by Liljefors (1970) In the two latter studies there were some geographical restrictions with respect to the residence of the twin pair Lundman took his twin sample from several towns in the central part of Sweden and Liljefors obtained his DZ pairs from 10 counties in central Sweden There were no such restrictions in the selection of twin pair in the present study Furthermore the mean age of the present twins was 60.7 years which is about 3 years higher than in Liljefors' study and about 10 years higher than in Lundman's a circumstance which might also tend to lower the response rate

In population studies concerned with a particular disease it has been recognized that persons with the disease in question are more likely to participate than others (Cobb et al 1957) The twin pair in the present investigation were accordingly not informed that the primary intention was to study IHD They were told that this was a general health check-up constituting part of the research program on the Swedish Twin Registry and furthermore that only the surviving co-twins in death discordant pairs were included

A non-participation group is often thought to differ in various ways from the group examined In a population study of 973 50-year-old men (Tibblin 1967) an analysis of the non-participant (Tibblin 1965) failed to show that they were more sick than those examined It was also found that the chief reason for non-participation (38 %) was a negative attitude to medical care in general In the present investigation the largest group of non-response (22 out of 57) has been labelled 'refusal' for other reasons This includes 8 persons who frankly declared a negative attitude to doctor, hospital and health check-up The rest of this group (16 persons) gave no reason for their refusal

As regards sex and zygosity there are some differences between the respondent and non-respondents The male MZ twins clearly show a greater willingness to participate than the DZ male and this reading seems to be independent of the partner's cause of death There is no such difference among the female twins The male twins who did not participate were also on average one year older than the respondents It is of course difficult not to say impossible to tell whether the observed differences are an influence a valid comparison between the zygosity groups However the prevalence of IHD as estimated from an earlier questionnaire did not reveal any substantial differences between the respondents and non-respondents

II METHODS

Time schedule for the examinations

The first co twins to be examined were seen in June 1971 and the last 26 months later in August 1973

The twin arrived at about 8.00 a.m. for the examination which was completed by 4.00 p.m. on the same day. One or two twins a day were examined. The procedures were undertaken according to a fixed time schedule.

Questionnaire and interview

The twins were all interviewed by the author in accordance with special questionnaires including questions on earlier diseases, hospital treatment and medication as well as questions on sociologic data, smoking habits, use of alcoholic beverages. The questions on cardiovascular symptoms followed a questionnaire designed at the London School of Hygiene and Tropical Medicine (Rose, 1962). The questionnaire on respiratory symptoms was based on that worked out by the British Medical Research Council (1960).

Physical examination

A routine clinical bedside examination was performed on every twin supplemented with the following examinations:

Blood pressure measurement

The blood pressure was measured in supine position by the author once at the beginning of the examination (caval blood pressure) and then after at least 15 minutes rest (basal blood pressure). A mercury manometer was used with a sleeve measuring 13 x 40 cm and the standard procedure recommended by WHO (1968) was followed. The blood pressure was read to the nearest 5 mm Hg. The systolic pressure level was determined by the first perception of sound. The diastolic fourth phase level was recorded when the sounds were fully muffled and the diastolic fifth phase when the sound disappeared.

Anthropometry measurement

Weight was measured to the nearest kilogramme with the subject

dressed in undershorts

Height was measured to the nearest centimeter with the subject barefooted and his back against the wall

Skinfold thickness was measured with a Harpenden caliper (Edward et al 1955) giving a pressure of 10 g/mm² in the subscapular and triceps area on the right side of the body. Two measurements were performed at each site and the mean was recorded to the nearest 0.2 mm

Relative weight was determined according to a weight/height index
$$\frac{\text{weight (kg)}}{\text{height (cm)}^2} \times 100$$

Arm circumference was measured to the nearest 0.5 centimetre at the mid of the pendant unclad right upper arm

Electrocardiographic examination

ECG were recorded at rest and for nearly all of the subjects also during and after an exercise test on a bicycle ergometer. The recording was made after about 10 minutes rest in supine position before exercise and with the following leads: I II III aVR aVL aVF CR₁ CR₂ CR₄ CR₅ and CR₇. During exercise with the subject sitting on a bicycle registrations were performed after 2 4 5 and 6 minutes at each load with the leads CR₂ CR₄ CR₅ and CR₇ (Holmér & Strandell 1961). The exercise test started generally at a load of 300 kilopond-meter/min (kpm/min) for both men and women. The load was increased stepwise after the completion of each 6 min period for men by 300 kpm/min and for women by 150 kpm/min. 1 3 and 10 minutes after exercise ECG was again recorded in supine position with the following leads: I II III CR₁ CR₂ CR₄ CR₅ CR₇.

A direct writing 4 channel electrocardiograph was used on the subject examined in Stockholm (Mingograph 42 Elema-Schönanander AB Solna Sweden); those in the province were mostly examined with a direct writing 6 channel electrocardiograph (Mingograph 61 Elema-Schönanander AB Solna Sweden). A mechanically braked bicycle ergometer (Monark Cyklostation AB Stockholm Sweden) was used the same for all the subjects but none examined in Karlstad where an electro-dynamically braked bicycle ergometer was used (Elema-Schönanander Solna Sweden). The paper speed for the registrations was 50 mm/s both before and after the test and also during the 5 min registrations at each exercise load. The other recordings during exercise were performed with a paper speed of 25 mm/s. The heart rate was calculated from the ECG.

The test was stopped when the patient was unable to continue due to fatigue dyspnoea angina pectoris or marked ST segment depression or serious arrhythmia. In some cases the test was discontinued earlier for orthopaedic reasons.

Working capacity The maximum working capacity ($\dot{V}_{O_{2\max}}$) was calculated according to a formula of Strandell (1964). To the greatest load the subject was able to perform for 6 minutes (\dot{V}_{O_2}) was added $n \times \dot{V}_{O_2}/6$ kpm/min where n is the number of minutes at the final load and \dot{V}_{O_2} the difference between the two highest loads.

Coding The ECGs were all coded by the author according to a modified system (Åstrand et al. 1967) of the original Minnesota Code (Blackburn et al. 1960).

The codings were made by the author over a period of about one month after completion of the study. All the ECGs were checked twice and some were also interpreted by an independent observer (Dr. T. Lundman).

Radiologic examination

Radiologic examination of heart and lungs was performed on every subject. The exposures were taken with the subject erect and in two planes (frontal and lateral). The tube-film distance for both positions was 1.5 m at all examinations. An experienced radiologist (Dr. G. Skogsberg) examined all the films and determined the total and relative heart volume (Jonsell 1939).

Blood investigations

At about 8.00 a.m. venous blood samples were drawn after about 12-hour fast and thirst. All the analyses on blood from the subjects examined in Stockholm were made at the Department of Clinical Chemistry, Serafimer Hospital. In the case of subjects examined outside Stockholm serum was obtained and transported in thermoses with carbolic acid snow for later analysis of cholesterol, triglycerides and uric acid at the Department of Clinical Chemistry, Serafimer Hospital.

Cholesterol was determined in an autoanalyzer according to the modified method of Levine & Zak (1964).

Triglycerides were determined in an autoanalyzer by the method of Kobl & Campbell (1970).

Uric acid was determined in an autoanalyzer by the method of Hawk et al. (1954).

The methods were checked continuously throughout the study by
 d terminations on standard serum

The other blood test included fasting blood sugar hemoglobin
 venous hematocrit erythrocyte sedimentation rate (E S R) as well
 a qualitative tests for urine protein and urine glucose The determina-
 tions were made at the hospital in question in accordance with the
 routine methods used there

Statistical method

Continuous and discrete variable among the surviving co-twins were
 compared according to the scheme in Fig 6

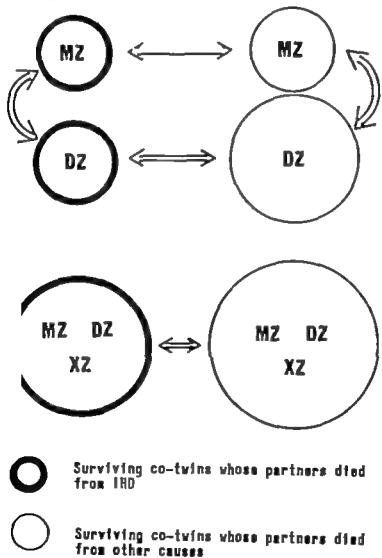


Fig 6 Statistical comparisons between the various group of surviving co-twins

Conventional methods were used for the calculation of mean values (M) and standard error of the mean (SE). Significance of differences between mean values was tested by Student's t-test. Differences between proportions were tested in fourfold tables by Fisher's exact probability test. Differences between combinations of risk factors (Chapter III, section 4) were tested in two-by-k tables using the chi-square test.

Intra pair difference (the death discordant pairs) of qualitative variable such as smoking and registered abuse of alcohol (Chapter III, section 3) were examined by the use of tables of binomial probability distribution (McNemar, 1955). If the direction of the distribution could be expected the one tailed probability was calculated. Degrees of significance were tested at the levels of 5 % ($p < 0.05$), 1 % ($p < 0.01$ **) and 0.1 % ($p < 0.001$ ***).

III

RESULTS

1 ISCHAEMIC HEART DISEASE AMONG THE SURVIVING CO-TWINS

This section deals with the prevalence of angina pectoris, myocardial infarction and electrocardiographic changes suggestive of IHD among the surviving co-twins.

Material and Method see Chapter I and II

Questionnaire and ophthalmic findings

The distribution of angina pectoris as recorded by interview according to the questionnaire proposed by Ros (1962) and that of myocardial infarction verified at hospital are given in Table 8.

Table 8 Distribution of angina pectoris and myocardial infarction (absolute numbers and prevalence rates) among respondent co-twins according to cause of death of index twin

		IHD				not IHD			
		ME	DX	XZ	Tot	ME	DX	XZ	Tot
		No	%	No	%	No	%	No	%
Male	Angina pectoris	1	10	2	11	3	7	-	-
	Myocardial infarction	3	30	3	12	1	4	1	2
	Total number	10		5		5	42	1	68
Female	Angina pectoris	2	25	1	11	-	-	4	8
	Myocardial infarction	-	-	-	-	-	-	1	2
	Total number	8		9		30	48	5	80

The prevalence of angina pectoris and myocardial infarction was higher among the male co-twin whose partner had died from IHD (7 % and 15 % respectively) than among those whose partner had some other cause of death (1 % and 3 % respectively); among the zygosity groups the highest prevalence rates were found for the MZ co-twins whose partners had died from IHD (10 % and 30 % respectively). Among the female subjects the prevalence of angina pectoris was likewise higher for the co-twin whose partners had died from IHD (18 %) compared to those whose partner had died from other cause (5 %). None of the differences was statistically significant. Only one (1 %) of the females had had a myocardial infarction verified at hospital.

Comment

The diagnosis of angina pectoris established by interview according to the questionnaire by Rose (1962) has been found to have a high specificity and also a fairly good sensitivity when compared to clinical judgement (Rose 1962; Hydn et al 1971). However some patients diagnosed as having angina pectoris on normal coronary arteriograms (Likoff et al 1967; Hump et al 1967); this has been found especially among premenopausal women (Fowler 1974). The reported prevalence of angina pectoris is about the same in men and women in several studies (Lannel et al 1961; Kannel & Feinleib 1971; Bengtson 1973) but the prognosis for survival and prognosis for more serious coronary manifestation is particularly discouraging in men. The Framingham study showed that on an average 4 men with angina pectoris expect a coronary attack within 5 years compared to half the risk for women (Kannel & Feinleib 1972). With regard to myocardial infarction the prevalence is considerably higher among men (Damber et al 1957; Fipstein et al 1965; Hagrup & From Hansen 1968).

In the present study the prevalence of angina pectoris is higher among the women than the men while the reverse is true of myocardial infarction. The surviving male co-twin whose partner died from IHD, display the highest prevalence of symptoms suggestive of manifest IHD and the rate is especially high among the MZ co-twin. This tendency is more marked for myocardial infarction verified at hospital. The amenability applied to the women as regards angina pectoris. None of the tendencies, however, gave statistically significant differences. Owing to the relatively low prevalence of manifest IHD and the small number of MZ and DZ co-twins whose partner died from this cause it is difficult to establish a significant of heredity in

manifest IHD. It has been said (Epstein 1964) that manifest IHD represents only the tip of the iceberg and that the additional cases hidden beneath the surface account for the relative insensitivity of diagnostic instrument. However, ECG during and after exercise is a relatively sensitive instrument for the detection of silent forms of IHD and is probably the best method in this respect for epidemiological studies (Blomqvist 1971; Helfant et al 1973; Åstrand 1973).

ECG and X-ray findings

The distributions of certain ECG findings at rest and during or after exercise are shown in Table 9 and 10. The findings have been tabulated according to the cod numbers in the modified Minnesota code (Åstrand et al 1967). ST depression, T wave negativity and ectopic beats were not coded in the 15 subjects who were on digitalis therapy. Resting ECG was recorded in all the co-twins but exercise tests could not be performed in 11 for various reasons (Table 11). The means for maximum working capacity (V_{max}) and final heart rate in the co-twins not treated with digitalis are given in Table 12. Both parameters are lower on average for the co-twin whose partner had died from IHD compared to those whose partner had died from other causes. Among the male MZ co-twin, however, both V_{max} and final heart rate are higher for those with partners who died from IHD while among the male DZ co-twins they are higher for those whose partner died from other causes than IHD. The difference was not statistically significant. Part of the intra-pair difference is probably due to the differences in age. Table 13 shows the mean values for relative heart volume among the surviving co-twin. For technical reasons and/or pleural change, no data are available for 3 female and 1 male DZ co-twins whose partner died from other causes than IHD. The male MZ co-twins whose partner died from IHD have larger relative heart volume than their male DZ counterparts ($p < 0.05$) but the pooled male syzygosity groups (IHD vs not IHD) do not differ. On the other hand, the pooled female syzygosity group (IHD vs not IHD) differ significantly ($p < 0.01$). The female MZ co-twins with partners who died from IHD have significantly ($p < 0.05$) larger relative heart volume than the MZ co-twins whose partner died from other causes. There is a similar tendency among the DZ females but the difference is not significant.

Table 9 Distribution of electrocardiographic changes at rest among respondent co-twins according to cause of death of ind x twin

	ECG code	Male					Females					
		M7	IHD MZ	X7 Tot	M7	not IHD M7 X7 Tot	MZ	IHD MZ XZ	Tot	MZ	not IHD MZ XZ Tot	
Q-waves	1:1	1	2	3	1	1	2			1	1	2
	2:1	3	1	4		4	6			1	1	2
	3								2		3	3
Axi d vlation left	2:1		4	4			4					
								1	1	1	4	5
High ampl R wave left type	3:1	2	2	2	1		3			1	2	4
	3	1	3	6		3	3			1	3	4
ST depressed n	4:1											
	2	1		1	3		3					4
	3	2	3	7	1	4	5			1	4	14
	4+5		7	7	3	4	7			4	5	16
	6+7		1	1	1		1					
T wav	3:1											
	1 to 5 mm					1	1				1	1
Flat or diphasic	3	1		1	2	1	3			1	3	4
Low amplitude	4	3	16	3	13	21	34			5	12	35
LUBB	7:1					1	1			1	1	1
Ectopic beats												
V ntrio	10:1-5	1	1		1	3	4			1	5	7
suprav ntrio	6 8		1	1							1	1
Digitalis therapy		2	2	4	3	2	5			2	3	4
Total examined		10	25	5	40	25	42	1	68	8	30	80

For Q wave and M7 & pr ion see appendix

Tabl 10 Distribution of the most pronounced I troardi graphi change during rest and r x rol e among repondent twins according to au f d nth of index twin

ST d pr	in	FCG de	Mal			not IHD			IHD			Female			n t IHD		
			IHD			IX			IX			Tot			MZ		
			MZ	DX	XE	Tot	MZ	DX	IX	Tot	MZ	DX	IX	Tot	MZ	DX	IX
ST d pr	in	4:1	2	4	6	3	1	4	8	3	3	3	9	2	11	13	4
			3	6	10	3	8	11	23	3	3	3	9	6	11	13	1
		2	4	4	8	3	10	13	23	3	3	3	9	6	7	16	23
		4+5 6+7	4	4	8	12	2	4	1	7			1	1	4	4	8
T wave			2	1	3	6	3	6	9			3	3	3	3	3	6
		5:1	1	1	1	1	1	1	4							1	1
		-1 to 5 mm	1	1	3	3	1	1	2	4					3	4	9
		Flat or diphasi	1	2	3	1	1	1	1	2	2	3	3	8	15	25	40
E t pi beat		Low amplitude	3	1	4	17	11	27	1	39							
		freq ventrio	10:1	1	1	1	4	5	6	12	1	1	3	1	1	6	1
		ca ion ventrio	5	4	1	9	4	5	9	18	4	4	4	4	3	9	12
		suprav ntri	6-8	2	2	4	1	3	4	8				2	2	5	7
Total number			8	3	4	35	19	39	1	59	6	9	15	27	43	1	73

For Q wave and ST d pr esions app ndix

Table 11. Distribution of response at co-twins not performing the ex-rcise test according to reason for non performance

	Males				Females			
	IHD		not IHD		IHD		not IHD	
	MX	DZ	XX	Tot	MX	DZ	XX	Tot
Statutory cardiac infarction	1			1				1
Cardiac insufficiency								1
Parkinson disease	1	1						
Stenotric post-polio myelitis			1	1				1
Rectal cancer			1	1				2
Orthopaedic reasons			1	1				
Refusal				1				
Total number	1	1	3	4				3

Table 1. Distribution of means for V_{max} and final heart rate at birth and final heart rate at death of individual twin (subject number in digit 11th copy omitted)

	Male										Female									
	IHD					not IHD					IHD					not IHD				
	MZ (SE)	DZ (SE)	XZ (SE)	Tot (SE)	MZ (SE)	DZ (SE)	XZ (SE)	Tot (SE)	MZ (SE)	DZ (SE)	XZ (SE)	Tot (SE)	MZ (SE)	DZ (SE)	XZ (SE)	Tot (SE)	MZ (SE)	DZ (SE)	XZ (SE)	Tot (SE)
V_{max} (lpm/min)	647 (91)	61 (37)	675 (95)	633 (33)	626 (45)	692 (30)	150 (0)	664 (6)	404 (6)	48 (49)	418 (37)	427 (16)	432 (21)	127 (16)	400 (0)	48 (13)	432 (21)	127 (16)	400 (0)	48 (13)
Final heart rate (bats/min)	158 (50)	148 (37)	158 (78)	152 (29)	150 (41)	157 (29)	146 (0)	155 (44)	149 (11)	153 (19)	154 (53)	153 (7)	158 (23)	153 (7)	118 (0)	153 (40)	158 (23)	153 (7)	118 (0)	153 (40)
Total number	8	3	4	35	19	30	1	59	6	9	15	7	45	1	73					

- Table 1. Distribution of means for heart volume among reproductive and non-reproductive twins according to age at death of individual twin

	Male										Female									
	IHD					not IHD					IHD					not IHD				
	MZ (SE)	DZ (SE)	XZ (SE)	Tot (SE)	MZ (SE)	DZ (SE)	XZ (SE)	Tot (SE)	MZ (SE)	DZ (SE)	XZ (SE)	Tot (SE)	MZ (SE)	DZ (SE)	XZ (SE)	Tot (SE)	MZ (SE)	DZ (SE)	XZ (SE)	Tot (SE)
Heart volume (c/m BSA)	449 (25)	384 (13)	462 (33)	410 (12)	413 (16)	407 (13)	520 (0)	411 (10)	430 (22)	397 (31)	412 (19)	368 (11)	368 (11)	353 (9)	400 (60)	260 (7)	368 (11)	353 (9)	400 (60)	260 (7)
Total number	10	5	5	40	5	41	1	67	8	9	17	30	45	1	77					

BSA=Body surface area

Comment

ST-T change have been omitted from the tables in the case of two anemic co-twins with a hemoglobin concentration below 10 g %. One of them a female DZ co-twin had been treated at hospital for hyperthyroidism and renal insufficiency and had a serum creatinin level of 3.6 mg %. Her partner had died from uraemia and the autopsy showed polycystic kidneys. The surviving co-twin has possibly also polycystic kidneys. The other a male MZ co-twin whose brother had committed suicide had signs of left ventricular hypertrophy with high amplitude R wave (3:1) and ventricular activation time >0.05 seconds. He also had an enlarged heart at X-ray with a relative size of 600 ml per square metre body surface. He was diagnosed as having a suspected cardiomyopathy. These two subjects showed no clinical signs of IHD and their ST depressions (4:1 2) were not considered indicative of IHD.

Two of the co-twins displayed physical and phonocardiographic signs of congenital heart disease and valvular heart disease respectively. One of them a male MZ co-twin whose partner had died from asthma bronchiale had dyspnoea on effort. He was treated with digitalis and the ECG showed intraventricular block. The probable diagnosis was atrial septal defect. The other co-twin a female DZ whose partner had died from malignant tumor of the breast revealed no symptoms of cardiac insufficiency. The ECG was normal. The probable diagnosis in this case was mitral insufficiency.

Grading of IHD

The cumulative distribution of different manifestations of IHD is given in Table 14. Myocardial infarction verified at hospital has been taken as the hardest criterion of IHD. Angina pectoris and various ECG criteria are then successively added to myocardial infarction. Figure 7 shows the prevalence of some of the combinations. The surviving co-twin whose partner died from IHD display a consistently higher prevalence of the various IHD manifestations than the whose partner died from other cause than IHD. This applies to both the male and the female groups but is somewhat more pronounced for the male particularly when the assessment of IHD has been extended to include ST depressions. The difference between the male co-twins (MZ + DZ + XZ) whose partner did or did not die from IHD is statistically significant ($p < 0.05$) not only when ST depressions >1 mm are included but also for ST depressions >0.5 mm. Comparing the prevalence of the various IHD manifestation within the zygosity group it is the MZ co-twin whose partner died from IHD who are consistently the

Table 14 Cumulative distribution of different IID manifestations among r pond nt twins = ording to cause of death of individual twin (good number = within parenthesis)

	Male						Female					
	IID			not IID			IID			not IID		
	MZ	DZ	XE	Tot	MZ	DZ	MZ	DZ	XE	Tot	MZ	DZ
Myocardial infarction	3	3		6	1	1						1
+ Angina pectoris	3	4		7	1					3		4
+ Q-wave (1:1:2)	4	4		8	2	3				4		4
+ ST depression (4:1:2)	7	13	1	21	6	12				10	15	16
during/after exercise												2
+ T wave (5:1:2)	7	13	1	21	6	12				10	15	16
during/after exercise												2
+ Q wave (1:1:3)	7	13	1	21	7	15				11	15	17
+ ST depression (4:1:3)	9	17	3	29	9	23				16	21	32
+ T wave (5:1:3)	9	17	3	29	9	23				16	21	32
+ LBBB (7:1)	9	17	3	29	9	23				16	21	32
Total number	10	25	5	40	25	42				17	30	48

For Q wave and ST depression see appendix

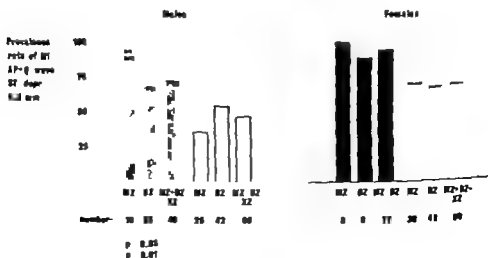
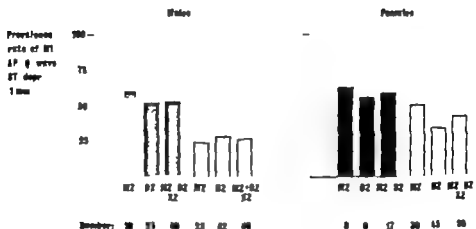
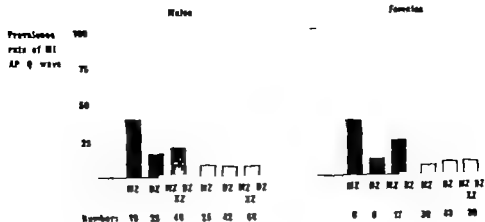


Fig 7 Prevalence rate of some IHD manifestations among respondent twins. Dark columns indicate that the partner died from IHD and white columns that partner died from other cause.

most affected group. They have significantly higher prevalence rates of IHD manifestation than their counterpart who's partners did not die from IHD. The difference is significant at the 5% level when ST depressions ≥ 1.0 mm are included and at the 1% level when ST depressions ≥ 0.5 mm are included. The female co-twins seem to be proportionally more affected than the males when, after IHD criteria are included irrespective of the cause of death (IHD/not IHD). The difference between the pooled female sib-pair group (IHD vs not IHD) is also significant ($p < 0.05$) when ST depressions ≥ 0.5 mm are included.

Comment

The clinical entities myocardial infarction, angina pectoris and sudden death are usually included in the definition of IHD. Most epidemiological studies also include various ECG findings in order to detect preclinical cases of IHD.

Concerning Q wave Q_1 is nearly always suggestive of old infarction. The specificity of Q_{1-2} as an indicator of IHD has been validated by autopsy and found to be high (Bjurulf et al 1967). All the pre-natal co-twins with myocardial infarction had Q_1 or Q_2 . In several population studies Q_{1-2} (Epstein et al 1965; Rowe 1971; Tibblin & Wilhelmsen 1971; Bengtsson 1973) and sometimes Q_{1-3} (Punsar & Karvonen 1973) have been taken as evidence of the probable presence of IHD. However, in a five year follow-up of nearly 13,000 men from seven countries the presence of a Q_3 had no significant prognostic power for future IHD (Blackburn et al 1970).

ECG changes, mainly ST change in connection with exercise are considered to be useful for detecting preclinical forms of IHD. It must be remembered however that some subjects with clinically documented IHD display no ECG abnormality at exercise just as there are others with abnormal ECG response at exercise and normal coronary angiography (Redwood & Epstein 1972). ST segmental depression in response to exercise has been shown to be a good predictor of overt IHD (Mattingly 1962; Åstrand & Lundman 1968; Blackburn et al 1970; Doyle & Kinch 1970). The order of ST segmental depression is also directly dependent on the relative load at exercise (Blomquist 1965). A study by Dean et al (1965) showed that compared to Master's two step test a near maximal test increased the sensitivity nine times in detecting ischaemic ST depressions.

ST depressions seem to be a less specific sign of IHD in women (Björck 1946; Lepehkin 1958; Åstrand 1965). Åstrand noted that the frequency of ST depressions ≥ 0.5 mm among men should be about

20 % at 55 years of age and about 35 % at 60. For females the expected frequency should be about 50 % at 55 years and over. These figures are based upon submaximal work load with fixed target pulse for different age-groups (Åstrand et al 1967). In the present study too ST depressions were more common among the women.

When ST depressions were included in the criteria suggesting IHD, the surviving male co-twins with partners who died from IHD displayed these signs of IHD significantly more often than those whose partners died from other causes. These findings go in the same direction as those of Liljefors (1970) for 91 male twin pairs. An extension of the ECG criteria of IHD to ST depressions ≥ 0.5 mm among females also significantly ($p < 0.05$) discriminates co-twins whose partners died from IHD from those whose partners died from other causes. In some studies (Epstein et al 1965; Bengtsson 1973) ST depressions have not been coded in the presence of high amplitude R wave (3:1) because in such cases ST depression were considered to reflect a hypertensive disease. If this procedure had been adopted in this study the significant difference ($p < 0.05$) in IHD manifestation between the surviving female co-twins would have been non-significant but the significance recorded both in the male co-twin would have been unchanged. However, experience from the Framingham study has shown that left ventricular hypertrophy (including ST depression and T-wave inversion) predicts the IHD incidence over and above what could be accounted for by the concurrent hypertension (Kannel et al 1970; Dawber & Kannel 1972). They considered these abnormalities to reflect not only a hypertensive hypertrophy but also IHD.

The differences recorded in IHD manifestations certainly reflect a genetic influence which is furthermore underlined by the striking difference in IHD manifestations in spite of a reversed age factor between the male MZ co-twins whose partners did or did not die from IHD. The possible role of environmental factors is discussed in some of the subsequent chapters.

2 BIOMETRIC FACTORS ASSOCIATED WITH ISCHAEMIC HEART DISEASE AMONG THE SURVIVING CO-TWINS

ANTHROPOMETRIC VARIABLES

Obesity is usually defined in terms of weight and then compared to standard or norms for the entire population or expressed as relative weight. Another measure of obesity is skin fold thickness.

Obesity is generally considered to involve a substantial risk of IHD though the relationship is somewhat unclear. In the Tecumseh study the prevalence of IHD was independently correlated with overweight among men but not among women (Epstein et al 1965). The Framingham study has shown an association between overweight and an increased incidence of angina pectoris and sudden death but not myocardial infarction (Kannel et al 1967). Multivariate analysis of the data from the seven country study (Key et al 1972) showed that a measure of relative weight or obesity made a significant contribution to future IHD when the factors of age, blood pressure, serum cholesterol and smoking were comparable.

Gertler & Whit (1954) found no difference in weight between controls and men under 40 with previous myocardial infarction. The mesomorphic body build was however more common among the infarction group than among the controls. Foraman & Lindgärde (1958) studied a similar material of post infarction men under 56. They distinguished between two subgroups in the material, one with above average length, weight and turdine factor and the other with small such factors. In an autopsy study by Björulf (1959) on 110 patients aged 25-88, 19 of whom had myocardial infarction, the severity of coronary atherosclerosis was correlated to the size of the subcutaneous fat cells but not to their number and also to the muscle mass. It was concluded that the grade of coronary atherosclerosis was more dependent on environmental influence than on genetic disposition. The result from a study by Björntorp & Sjöström (1971) also suggests two forms of obesity. One is characterized by a hypertrophy of fat cells and is of a moderate degree. This type of obesity has been shown to be associated with metabolic disturbance. The other form has an increased number of fat cells and is associated with much more severe obesity. In a comparison between post infarction male and control, Bracht et al (1972) found no difference with regard to body fat and fat cell size. In a study in women Bengtsson (1973) reported no significant

difference with regard to overweight between control groups and those with myocardial infarction angina pectoris and ECG signs suggestive of IHD respectively. In another study (Granér et al 1966) on 173 males and 51 females no correlation was found between coronary angiographic findings and relative weight.

It must be born in mind however that obese persons are more likely to be hypertensive and hyperlipidaemic hyperglycaemic and hyperuricaemic and that this probably reflects chronic caloric imbalance in susceptible people (Stamler 1973).

Material and Method: see Chapters I and II

Results

The distribution of the means of some of the anthropometric parameters is shown in Table 15. There are no substantial differences among the males. The MZ co-twin whose partners died from IHD are somewhat heavier and have a bigger arm circumference as well as more skinfold fat in the triceps area than either the DZ co-twins with IHD partners or the MZ co-twins whose partners died of other causes than IHD. These differences are not statistically significant. The female co-twins especially the MZ group whose partners died from IHD are heavier and have somewhat more skinfold fat than the co-twin whose partners died from other causes than IHD but these differences are not statistically significant. The distribution of overweight co-twins defined as a relative weight ≥ 110 according to the earlier described weight/height index is shown in Table 16. Of the male co-twins whose partners died from IHD 18% are overweight compared to 4% of those whose partner died from other causes. The corresponding figures among the females are 53% and 46%. None of the differences with regard to the occurrence of overweight is statistically significant.

Conclusions

Height and skeletal measurements were found to be chiefly dependent on genetic factors in twin studies by O'Brien & de George (1959) and Takkum (1964). On the other hand Lundman (1966) and Liljefors (1971) in their twin study found weight and skinfold thickness to be dependent on both genetic and environmental factors. The co-twins whose partner died from IHD displayed a tendency to greater relative weight and skinfold fat compared to those whose partner died from other causes. This was most marked among the females especially the MZ co-twins. This finding could indicate that these parameters in some measure are linked to the development of IHD in females.

tbl 15 list built in time for anthropometric parameters of respondents and twins
 * of death if listed as twin

Male

	M=1				n t IID				IID				not IID					
	M7 (1)	M7 (4F)	X7 (4F)	T t (41)	M7 (4F)	DZ (4F)	XZ (81)	Tot (57)	MZ (SE)	M (SE)	DZ (SE)	XZ (4F)	Tot (SE)	MZ (SE)	M (SE)	DZ (SE)	XZ (SE)	Tot (SE)
A	173 (14)	173 (09)	17 (18)	173 (07)	173 (11)	171 (11)	170 (81)	1718 (08)	1569 (-)	1638 (15)	-	-	1605 (15)	1587 (11)	159 (09)	515 (20)	019 (07)	Tot (07)
B	756 (31)	7 (18)	(0)	731 (14)	19 (24)	7 (15)	64 (0)	719 (13)	701 (7)	713 (46)	-	-	708 (40)	6 (15)	661 (19)	760 (60)	648 (13)	Tot (13)
C	114 (18)	96 (06)	90 (14)	100 (06)	90 (07)	98 (04)	100 (0)	93 (04)	31 (19)	00 (0)	-	-	14 (14)	194 (10)	193 (07)	203 (33)	193 (03)	Tot (03)
D	139 (16)	150 (11)	158 (11)	137 (08)	139 (13)	163 (10)	13 (0)	161 (07)	40 (34)	14 (28)	-	-	-6 (-)	184 (13)	08 (12)	316 (26)	09 (09)	Tot (09)
E	314 (10)	300 (05)	301 (07)	304 (04)	300 (0)	304 (04)	80 (0)	30 (03)	30 (19)	317 (13)	-	-	318 (11)	98 (06)	303 (08)	333 (03)	30 (03)	Tot (03)
F	103 (43)	99 (4)	100 (25)	100 (19)	98 (3)	101 (17)	91 (0)	100 (16)	1 (83)	11- (73)	-	-	117 (54)	107 (33)	111 (8)	139 (160)	110 (2)	Tot (2)
Total	10	5	5	50	5	5	1	68	8	9	-	-	17	30	48	-	80	Tot (80)

A=Height, B=Weight, C=Skinfold thickness, D=Skinfold thickness, E=Arm circumference, F=Relative weight

Table 16 Distribution of respondent co-twins with overweight (relative weight ≥ 110) according to cause of death of index twin

	IHD				not IHD			
	MZ No %	DZ No %	XZ No %	Tot No %	MZ No %	DZ No %	XZ No %	Tot No %
Overweight male (relative weight ≥ 110)	3(30)	4(16)	0	7(18)	7(28)	9(21)	0	16(24)
Total number	10	25	5	40	25	42	1	68
Overweight female (relative weight ≥ 110)	4(50)	5(56)	-	9(53)	11(37)	24(50)	2(100)	37(46)
Total number	8	9	-	17	30	48	2	80

BLOOD PRESSURE

Elevated blood pressure has been designated one of the major risk factors for premature IHD (Atherosclerosis Study Group 1970; Simon 1970; Miller & Epstein 1972; Stamler 1973). Data from 14 years of follow-up of 5127 men and women in the Framingham study (Kannel et al 1971) have shown that the risk of developing IHD is directly proportional to blood pressure from the lowest to the highest level for both men and women. Furthermore, similar gradients of risk were observed whether persons were classified by their systolic or diastolic pressure, although the systolic pressure displayed a stronger association with the risk of developing IHD.

According to Epstein (1964), familial aggregations of IHD seem to be in addition to some extent by hypertension which could thus be one of the mechanisms of genetic transmission. A genetic influence has been demonstrated for essential hypertension in family studies and studies on twins extensively reviewed by Miall (1971) who supports the hypothesis that arterial pressures are determined polygenically. He also notes that familial factors account for only one third of the variance of systolic pressure and one fifth of that of diastolic and that non-familial environmental factors presumably account for the remainder.

Some of the twin studies by Osborne et al (1963), Downie et al (1969) and Liljefors (1970) have shown blood pressure to be pre-

dominantly under environmental influence as elucidated from comparisons between MZ and DZ intra pair variances whereas studies by Takkinen (1964) and Lundman (1966) have pointed to a stronger genetic influence. Genetic factor also seem to play a more important role in females to judge from the studies by Lundman and Osborne et al.

Material and Methods: see Chapters I and II

Results

The distribution of means for casual and basal blood pressure and pulse rates is shown in Tables 17 and 18. Casual and basal systolic pressures as well as diastolic phase 4 and 5 pressures are on average higher for the co-twins with partner who died from IHD than for the whose partners died from other cause. The difference are relatively marked among the females especially for casual systolic pressure where the difference is significant ($p < 0.05$). Comparisons by zygosity show that the male MZ co-twins whose partners died from IHD do play a slight tendency to have higher measured pressures than the DZ co-twin in this IHD group. For the females there is the reverse tendency for casual pressures the DZ co-twins having the highest value. The casual systolic pressure are also significantly ($p < 0.05$) higher for the female DZ co-twins whose partners died from IHD than the female DZ co-twins whose partners died from other cause than IHD. The distribution of co-twins with elevated blood pressure defined here as basal systolic ≥ 160 mm Hg and/or ≥ 95 mm Hg diastolic phase 4 is presented in Table 19. The occurrence of "hypertension" according to this definition is 48 % among the male co-twins with partners who died from IHD and 41 % among those whose partners died from other cause. The corresponding figures for the females being 59 % and 41 %. None of the differences is statistically significant.

Comment

Population studies concerning blood pressure extensively reviewed by Tibblin (1967) show that blood pressure rises with age that the systolic pressure increases more than the diastolic after 50 and that blood pressure in old women is higher than in old men. In the present investigation the blood pressure especially the casual systolic are surprisingly high in the female whose partners had died from IHD. To some extent this could possibly be explained by the fact that they were on average 4 year older as well as heavier than the female co-twins whose partner had died from other causes than IHD. It has been found

Table 17 Distribution of mean for a and b) and pressure (B p) and pulse rate in respondent co-twins according to cause of death of index-twin

	Males										Females									
	MZ (SC)	DZ (SC)	IX (SC)	Tot (SC)	MZ (SC)	DZ (SC)	IX (SC)	Tot (SC)	MZ (SC)	DZ (SC)	IX (SC)	Tot (SC)	MZ (SC)	DZ (SC)	IX (SC)	Tot (SC)	MZ (SC)	DZ (SC)	IX (SC)	Tot (SC)
A	167 (109)	5166 (59)	147 (68)	164 (47)	163 (54)	157 (34)	170 (0)	159 (8)	11 (1)	11 (1)	11 (1)	195 (68)	175 (71)	7 (1)	172 (49)	1 (25)	5174 (40)			
B	99 (44)	96 (0)	88 (46)	93 (19)	93 (23)	93 (15)	93 (0)	93 (1)	11 (1)	11 (1)	11 (1)	99 (9)	94 (8)	0 (0)	93 (23)	5 (125)	94 (2)			
C	98 (47)	93 (3)	88 (46)	95 (19)	91 (24)	92 (15)	93 (0)	92 (13)	11 (1)	11 (1)	11 (1)	98 (8)	90 (8)	0 (0)	91 (4)	7 (125)	92 (5)			
D	81 (45)	76 (29)	66 (38)	76 (23)	75 (29)	77 (23)	68 (0)	76 (18)	78 (53)	78 (3)	78 (48)	78 (34)	83 (33)	9 (24)	83 (24)	4 (190)	83 (5)			
Tot 10	5	5	5	40	25	42	1	68	8	9	9	17	30	48	2	80				

A=Systolic B p ; B=Diastolic B p phase 4; C=Diastolic B p phase 5; D=pulse rate beats/min

rding t au of d nth of ind = twin

M al										F mal									
IND					n t IND					IND					n t IND				
MZ	DX	IX	T t	Tot	MZ	DX	IX	Tot	MZ	DX	IX	T t	Tot	MZ	DX	IX	T t	Tot	
(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	(SE)	
A	154 (11 6)	5 (5 0)	147 (7 5)	8 (4 3)	147 (4 7)	0 (2 6)	5 (2 6)	0 (2 4)	143 (2 4)	172 (13 2)	5 (7 3)	166 (7 3)	1 (2 4)	169 (7 1)	132 (5 7)	3 (4 7)	154 (10 0)	1 (3 5)	154 (3 5)
B	97 (5)	0 (1 6)	91 (5 7)	8 (1 8)	90 (2 6)	58 (1 3)	8 (1 3)	0 (1)	89 (1)	94 (4)	4 (3 7)	89 (3 7)	-	91 (2 7)	8 (2 5)	88 (2 1)	11 (12 5)	3 (1 6)	88 (1 6)
C	95 (4 6)	0 (1 7)	85 (5 7)	0 (1 7)	89 (2 6)	6 (1 5)	5 (1 5)	0 (1 3)	88 (1 3)	93 (4 2)	8 (3 8)	88 (3 8)	-	91 (2 8)	2 (6)	87 (1)	0 (10 0)	1 (1 6)	87 (1 6)
D	75 (3 5)	0 (3)	62 (4)	8 (1 8)	71 (5)	8 (2 3)	2 (2 3)	0 (1 7)	73 (1 7)	70 (4 1)	8 (3 6)	68 (3 6)	-	69 (6)	4 (2 9)	76 (1 9)	3 (8 0)	7 (1 6)	76 (1 6)
Tot 10	25	5	40	25	42	1	68	1	68	8	9	9	17	30	48	2	80	2	80

A= Syst lic B p ; B Dia tolic B p phase 4; C= Dia tolic B p phase 5; D= Pulse rate beats/min

1

1

Table 19 Distribution of respondent co-twins with elevated basal blood pressure (≥ 160 mm Hg systolic and/or ≥ 95 mm Hg diastolic phase 4)

Elevated blood pressure	IHD								not IHD							
	MZ		DZ		XZ		Tot		MZ		DZ		XZ		Tot	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Males	6(60)		12(48)		1(10)		19(48)		12(48)		15(57)		1(100)		28(41)	
Tot	10		25		5		40		25		42		1		68	
Females	5(63)		5(56)		-		10(59)		16(53)		15(31)		2(100)		33(41)	
Tot	8		9				17		30		48		1		80	

(Humerfeldt 1963) that when age is held constant systolic blood pressure increases only about 3 mm Hg for each 10 kg increase in weight and diastolic blood pressure only 2 mm Hg. The twin studies mentioned earlier suggest that the genetic influence on blood pressure may increase with age and may be greater in women. The present co-twins whose partner died from IHD did play a slight tendency to higher blood pressure and the high t value occur in the MZ co-twins except in the case of a usual systolic and diastolic pressures in the females where the high t value came from the DZ co-twins. ECG signs indicative of IHD were found by Bengtsson (1973) to be fairly unreliable in this respect and it was concluded that in women they could probably be caused by hypertension. It is thus conceivable that the relatively high blood pressures recorded in the female co-twins whose partners died from IHD are partly responsible for the high prevalence of ECG signs suggestive of IHD.

LIPIDS

Cholesterol like hypertension has been designated one of the major risk factors for premature IHD (Atherosclerosis Study Group 1970; Simborg 1970; Stamler & Epstein 1972; Stamler 1973). The average serum cholesterol level for persons with myocardial infarction is higher than in the normal population (Björck et al 1957; Carlsson 1960; Gustafsson et al 1972) and several prospective studies have demonstrated strong statistical associations between elevated serum cholesterol and IHD in men (Key et al 1970; Rosenman et al 1970; Kannell et al 1971; Weitlund & Nicolay 1972). The Framingham study has shown that elevated serum cholesterol in women under 50 is associated

with an increased risk of developing IHD (Kannel et al 1971) Elevated cholesterol has also been found in women with manifest IHD (Mulcahy et al 1967)

Fewer reports have been published on serum triglycerides and IHD. Prospective studies have shown that elevated triglyceride are likewise associated with an increased risk of myocardial infarction (Carlson & Böttiger 1972; Tibblin 1972). Significantly higher serum triglyceride were found by Bengtson (1973) in women who had had myocardial infarction and in women with ECG changes suggestive of IHD as compared to the general female population. No significant over-representation of high serum triglyceride was found in women with angina pectoris.

Lipoproteins can be classified (Beaumont et al 1970) by means of ultracentrifugation (Gofman et al 1954) or electrophoresis (Fredrickson & Le 1965). Applying their lipoprotein phenotyping system to a large number of persons with a primary increase in plasma lipids, Fredrickson and co-workers managed to distinguish between five primary hyperlipoproteinaemias. Three of them (II-IV) are associated with an increased risk of IHD. Of the established hyperlipoproteinaemia the classical familial hypercholesterolaemia (type II a) is thought to be inherited by an autosomal dominant gene with a high penetrance; it can occur in a homozygous form (Fredrickson 1971; leading article Lancet 1971). The primary familial hyperlipoproteinaemias however usually include only the gross hyperlipidaemic cases which are fairly uncommon in the general population. In a recent study on 412 first degree relatives of 101 young survivors of myocardial infarction (Sikkinen & Aro 1973; Aro 1973) the mean levels of serum cholesterol and serum triglycerides were significantly higher than in a control population but lower than in the index patients. Hypercholesterolaemia (type II a) occurred 1.8 times, hypertriglyceridaemia (type IV) 1.3 times and combined hyperlipidaemia (type II b) 2.5 times more frequently in relatives than in controls. It was furthermore concluded that one-third of patients with premature IHD have a familial trait of hyperlipidaemia but it could not be established whether this trait is inherited or produced by environmental factors.

Some of the earlier twin studies have shown that the variability of serum cholesterol is due to both environmental and genetic factors (Osborne et al 1959; McDonough et al 1962; Meyer 1962; Jensen et al 1965; Riskind et al 1968). Comparisons between twins living together and apart showed a smaller intra-pair variance among the former indicating an influence from the environment. But as the intra-pair

difference was significantly greater for the DZ than the MZ pairs there seems to be a genetic influence too

However the study by Gedda & Poggi (1960) on twin pairs below the age of 20 showed that genetic factors strongly influenced the variability of cholesterol. In his study on 196 smoking discordant male and female twin pairs using the Swedish Twin Registry Lundman (1966) found cholesterol to be under both environmental and genetic influence while for triglycerides and phospholipids the genetic component was more obvious in the female pairs. Studying 91 male twin pairs Liljefors (1970) found evidence of a genetic influence on the cholesterol level from the significantly smaller variance in the MZ than the DZ twins. There was no such evidence of heredity in respect of triglycerides.

Material and Methods : see Chapters I and II

Results

The means for cholesterol and triglycerides are given in Table 20. Because the lipid values showed a skewed distribution they were transformed to their logarithms before making statistical comparisons. Both the cholesterol and the triglyceride values are higher on average for the male twins whose partners died from IHD than for those whose partners died from other cause. The difference is not statistically significant. The highest lipid value among the male co-twins is that of the DZ co-twins whose partner died from IHD. Among the female co-twins there is a similar trend for cholesterol with higher means for the co-twins with partners who died from IHD. The trend for triglyceride is the reverse of this mainly due to comparatively low values among the DZ co-twins whose partner died from IHD. None of the differences within the various male and female groups is statistically significant.

The distribution of co-twins with elevated cholesterol (≥ 50 mg/100 ml) and/or elevated triglyceride (≥ 150 mg/100 ml) is given in Table 21. Of the male co-twins whose partners died from IHD 60% had elevated cholesterol and/or triglyceride value compared to 50% of those whose partners died from other causes. Among the female co-twins the corresponding figures were 76% vs 68%. None of the differences is statistically significant.

Comments

Men and women often have much the same cholesterol level from 70-90

Male										Female									
ME		DE		IX		Tot		ME		DE		IX		Tot		ME		DE	
M	(SE)	M	(SE)	M	(SE)	M	(SE)	M	(SE)	M	(SE)	M	(SE)	M	(SE)	M	(SE)	M	(SE)
221	0.239	6	14	0.231	8	210	6.226	5	80	0.221	5	280	9.272	4	276	3	269	243	5
(8.4)	(11.8)	(20.0)	(8.1)	(9.2)	(8.0)	0	(6.1)	(35.2)	(21.8)	-	-	-	-	-	(19.5)	(9.6)	(8.7)	(17.5)	(6.5)
127	5.167	8	151	0.155	6	144	8.137	4	110	0.139	7	168	1.117	8	141	5	150	5.157	7
(17.8)	(18.9)	(27.4)	(13.2)	(14.1)	(11.1)	0	(8.5)	(28.2)	(9.9)	-	-	-	-	-	(15.1)	(15.0)	(17.8)	(30.0)	(12.0)
06	2.17	14	2.14	1	0.09	2.04	10	2.19	2.06	-	-	-	-	-	12	2.12	2.14	2.24	14
(0.07)	(0.04)	(0.10)	(0.04)	(0.04)	(0.03)	0	(0.02)	(0.07)	(0.05)	-	-	-	-	-	(0.04)	(0.04)	(0.03)	(0.08)	(0.02)
Tot 10	25	5	40	25	42	1	68	8	9	-	-	-	-	-	17	30	48	2	80

A=Chole t rol (mg/100 ml); B Triglyo ride (mg/100 ml); C=Log triglyoeride (mg/100 ml)

difference was significantly greater for the DZ than the MZ pairs there seems to be a genetic influence too

However the study by Gedda & Poggi (1960) on twin pairs below the age of 20 showed that genetic factors strongly influenced the variability of cholesterol. In his study on 196 smoking discordant male and female twin pairs using the Swedish Twin Registry Lundman (1966) found cholesterol to be under both environmental and genetic influence while for triglycerid and phospholipids the genetic component was more obvious in the female pairs. Studying 91 male twin pairs Liljefer (1970) found evidence of a genetic influence on the cholesterol level from the significantly smaller variance in the MZ than the DZ twins. There was no such evidence of heredity in respect of triglycerides.

Material and Method : see Chapters I and II

Result

The means for cholesterol and triglycerides are given in Table 20. Because the triglycerid values showed a skewed distribution they were transformed to their logarithm before making statistical comparisons. Both the cholesterol and the triglyceride values are higher on average for the male co-twins whose partners died from IHD than for those whose partner died from other causes. The difference is not statistically significant. The highest lipid values among the male co-twins are those of the DZ co-twins whose partners died from IHD. Among the female co-twins there is a similar trend for cholesterol with higher means for the co-twins with partners who died from IHD. The trend for triglycerides is the reverse of this mainly due to comparatively low values among the DZ co-twins whose partners died from IHD. None of the differences within the various male and female groups is statistically significant.

The distribution of co-twins with elevated cholesterol (≥ 30 mg/100 ml) and/or elevated triglycerides (≥ 150 mg/100 ml) is given in Table 21. Of the male co-twin whose partner died from IHD 60 % had elevated cholesterol and/or triglyceride values compared to 50 % of those whose partners died from other cause. Among the female co-twins the corresponding figures were 76 % vs 68 %. None of the differences is statistically significant.

Comments

Men and women often have much the same cholesterol level from 20-50

years of age but after that the values tend to be higher in female. The triglycerid level is usually lower in females than males. It is also well known that lipid level increases with age (Beaumont et al 1970). In the U.S.A. the average serum cholesterol level increases with age in both men and women until about 40 years when it levels off in men but continues to rise in women until about 60 years of age (Simborg 1970). In view of these findings it is possible that the difference in cholesterol values between the female co-twins whose partner died or did not die from IHD is to some extent a reflection of the age difference (4 years). The twin study by Lundman (1966) showed only a weak association between age and serum lipid level in males but highly significant correlations in females especially among the MZ twins; the subjects however were on average about 10 years younger than the twins in the present investigation. Triglycerid showed the same trends as cholesterol among the male co-twins but there was a trend among the female. However it must be born in mind that one of the differences could be due to random variation because the group were relatively small especially the female IHD group.

The cut off points used here to denote elevation of cholesterol and/or triglycerids have been chosen fairly liberally. It is however well known that there is no evidence of a critical level for these factors. In the Poling Project in the U.S.A. men with a cholesterol value over 250 mg/100 ml carried a 10 year IHD risk about twice as high as the remainder of the population (Stamler & Epstein 1972). The Stockholm Prospective Study (Carlsson & Böttiger 1972) also shows a linear relationship to risk over the entire range of triglycerid and cholesterol concentrations.

DIABETES MELLITUS

The association between diabetes mellitus and IHD has long been recognized. An increased incidence of IHD is found not only in persons with overt diabetes mellitus but also in those with impaired glucose tolerance (Ostrand et al 1965; Epstein 1967). The Framingham data show a higher incidence of IHD in diabetic women than in diabetic men (Kannel et al 1967). Conversely a high proportion of subjects with IHD were found to have diabetes mellitus (Siever et al 1961; Siever 1963; Wahlberg 1966; Simborg 1970). Survivors of myocardial infarction had abnormal intravenous glucose tolerance more often than controls (Wahlberg 1966). There seems to be general agreement on the hereditary

character of diabetes mellitus (Gedda & Brenchi 1970) although this has been difficult to assess in population studies (Epstein 1964). However twin studies have shown considerably higher concordance regarding diabetes mellitus in MZ pairs compared to DZ pairs (Therberg 1938; von Verschuer 1938; Joslin et al 1959; Harvald & Hauge 1965). In a study on MZ twins discordant with respect to overt diabetes mellitus Cerasi & Luft (1967) found a closely similar insulin response after glucose infusion indicating a genetic influence.

Material and Method : see Chapters I and II

Results

The prevalence rates for diabetes mellitus diagnosed earlier and the combination of this and fasting blood sugar >100 mg/100 ml are about the same for the male co-twins irrespective of the cause of death of the partner (IHD/not IHD) (Table 22). The prevalence of overt diabetes mellitus is especially high among the MZ co-twins whose partners had not died from IHD (20%) and it differs significantly ($p < 0.05$) from the prevalence (0%) among the DZ co-twins in the same group. However it is worth noting that the 5 MZ co-twins whose partners died from other cause than IHD who had diabetes mellitus were all concordant in this respect i.e. their deceased partner had also had overt diabetes mellitus but this was not the cause of death in any of these.

Table 22 Prevalence of diabetes mellitus and/or fasting blood sugar >100 mg/100 ml in surviving co-twins according to cause of death of index twin

	IHD				not IHD				Total
	MZ No	DZ No	MZ %	Total No	MZ No	DZ No	MZ %	Total No	
Males									
A	1	2	100	3	4	0	0	4	74
B	1	4	100	5	5	2	48	7	103
Tot	10	5		40	25	42		1	68
Females									
A	1	1	125	2	2	4	83	6	75
B	2	1	250	3	3	4	83	7	88
Tot	8	9		17	30	48		2	80

A=Diabetes mellitus; B=Diabetes mellitus and/or blood sugar >100 mg/100 ml

ca Th female co twins whose partner died from IHD have somewhat higher prevalence rates than the co twins whose partner died from other cause. None of the differences among the females is statistically significant. Diabetes mellitus was the cause of death in two of the deceased twins in the present material of death discordant pairs. One of their surviving co-twins (female DZ) also had overt diabetes treated with tablets. Her fasting blood sugar was 187 mg/100 ml. The other (male DZ) displayed no signs of diabetes mellitus having a fasting blood sugar of 81 mg/100 ml and no glucose in the urine.

Comments

In the present investigation overt diabetes mellitus and elevated fasting blood sugar do not appear to be responsible for the significantly higher prevalence of IHD manifestations among the male co twins whose partner died from IHD compared to those whose partner did not die from IHD because the prevalence of overt diabetes mellitus and elevated fasting blood sugar was about the same in both groups. With the twin study by Liljefer (1970) indicates that the presence of diabetes mellitus is a determining factor for the occurrence of IHD in twins who probably had IHD in the IHD discordant pair. However, in other studies an overrepresentation of diabetes mellitus has been reported among cases with IHD (Sievers et al 1961; Sievers 1963; Wahlberg 1966; Simborg 1970). That genetic factor of importance for the occurrence of diabetes is underlined by the fact that 5 male MZ pairs (not IHD death discordant) were concordant with respect to overt diabetes mellitus. The prevalence of diabetes mellitus also seems rather high among the surviving co twins whether or not their partner died from IHD compared to the general population (Grønberg et al 1967; Bengtsson 1973).

URIC ACID

Clinical and epidemiological studies have shown that hyperuricemia is more common in IHD subjects than in others (Gertler & Whit 1954; Myer et al 1968). Elevated uric acid has also been associated with an increased future risk of developing IHD (Kannel et al 1967).

The possible etiologic role of uric acid in the development of IHD is not clear. Gertler & Whit (1964) suggested that uric acid could increase the adhesion of lipid to arterial wall. It has also

been proposed that uric acid influences thrombocyte aggregation (Newland 1968). Studies by Theorell (1971) have shown that uric acid production is high in persons who have had myocardial infarction and that after the infarction the subjects do not regulate their renal excretion of uric acid as efficiently as do healthy subjects.

Twin studies have documented an influence of hereditary factors in the variation of uric acid levels (Harvald & Hauge 1955; Jensen et al 1965; Liljefors 1970).

Material and Methods: see Chapters I and II

Results

The mean value for uric acid in the surviving co-twins with and without diuretic treatment are presented in Table 23. None of the differences between means among males and females respectively is statistically significant; this applies both to those with and to those without diuretics.

The distribution of co-twins with elevated uric acid (≥ 6 mg/100 ml) is given in Table 24 which includes those on diuretic therapy. Of the male co-twins whose partner died from IHD 50 % have elevated uric acid compared to 29 % of those whose partners died from other cause. With the exception of one MZ co-twin whose partner had not died from IHD all the male co-twins on diuretic therapy have elevated uric acid. Among the females the corresponding figures are 18 % compared to 10 %; out of 18 female co-twins on diuretic therapy 5 have elevated uric acid (2 MZ with IHD partner and 3 DZ whose partners died from other causes). The difference in the occurrence of elevated uric acid are not statistically significant.

Comments

As a larger proportion of the subjects whose partner had died from IHD were on diuretics the analyses of the results are of course biased. When the co-twins on diuretic therapy are excluded only slight differences are found among the others. The female MZ co-twins with partner who had died from IHD have higher uric acid levels on average than either the DZ twins in the same IHD group or the MZ twins whose partner had not died from IHD; to some extent this may be due to difference in weight as there is known to be a positive correlation between uric acid and obesity (Myers et al 1968).

The difference in the occurrence of elevated uric acid are mainly due to the inclusion of persons receiving diuretic treatment, so that

Tabl 23 Di tributi n f mean for uri acid in re pond nt o-twin with and without diur tic tr at
ment A rding t au f d ath f ind x-twin

	Male										Female									
	IHD					n b IHD					IHD					not IHD				
	MX (SE)	DX (SB)	XE (SB)	Tot (SB)	M (SB)	MX (SE)	DX (SE)	XE (SB)	Tot (SB)	M (SB)	MX (SB)	DX (SE)	XE (SB)	Tot (SB)	M (SB)	MX (SE)	DX (SB)	XE (SE)	Tot (SE)	M (SE)
No diuretic th rapy:																				
Uri acid (mg/100 ml)	5 5 (0 2)	3 4 (0 3)	6 8 (0 7)	5 6 (0 2)	5 6 (0 2)	5 2 (0 3)	5 5 (0 2)	5 7 (0 0)	5 4 (0 1)	5 4 (0 1)	5 3 (0 3)	4 8 (0 2)	5 0 (0 2)	4 2 (0 2)	4 7 (0 2)	5 5 (0 0)	4 7 (0 2)	5 5 (0 0)	4 5 (0 1)	5 4 (0 1)
Number	7	23	5	35	43	42	1	66			5	7	12	24	42	1	67			
Diuretic th rapy:																				
Uri acid (mg/100 ml)	6 4 (0 1)	6 9 (0 7)	6 6 (0 3)	4 6 (1 5)	-	4 6 (1 5)	-	4 6 (1 5)	-	-	7 4 (0 8)	4 8 (0 0)	6 3 (0 8)	4 8 (0 3)	6 0 (0 3)	6 0 (0 0)	6 0 (0 0)	5 5 (0 3)	5 5 (0 3)	5 5 (0 3)
Number	3	2	5			3	2	5			3	2	5	6	6	1	13			

Table 24 Distribution of respondent co-twins with elevated uric acid (≥ 6 mg/100 ml) according to cause of death of index-twin

Elevated uric acid (≥ 6 mg/100 ml)	IHD				not IHD			
	MZ No (%)	DZ No (%)	XZ No (%)	Tot No (%)	MZ No (%)	DZ No (%)	XZ No (%)	Tot No (%)
Males	5(50)	11(44)	4(80)	20(50)	8(32)	12(49)	0	20(29)
Tot	10	25	5	40	25	42	1	68
Females	3(38)	■	-	3(18)	0	7(15)	1(50)	8(10)
Tot	8	9	-	17	30	48	■	80

in this study the etiologic role of uric acid is difficult to establish. It seems reasonable to suppose however that an elevated uric acid level provoked by diuretic therapy could still act as a risk factor for IHD.

HEMOGLOBIN HEMATOCRIT AND ERYTHROCYTE SEDIMENTATION RATE

Elevated hemoglobin values have been associated with an increased risk of IHD in men (Davber & Karmel 1961; Böttiger & Carlsson 1972). In a follow-up study of 50 year old men in Gothenburg (Tibblin 1973) a high hematocrit level was associated with the incidence of both fatal and non fatal myocardial infarction but it also correlated with death from other causes than IHD especially cancer. In the Stockholm Prospective Study an elevated erythrocyte sedimentation rate (ESR) has also been associated with an increased risk of IHD (Carlsson & Böttiger 1972).

Material and Methods: see Chapters I and II

Results

Table 25 give the mean for hemoglobin hematocrit and erythrocyte sedimentation rate (ESR). Both the male and the female values for hemoglobin and hematocrit are on average somewhat lower for the co-twins whose partner died from IHD than for those whose partner died from other causes although the differences are not statistically significant. With regard to ESR the opposite applies. The differences between zygosity groups are also only light and non significant.

Tabl 5 Distribution of mean for hemoglobin (Hb) in mono zygotic (MZ) and dizygotic (DZ) twins according to age of death, if individual twin (ZSR) in the population

Tot (ZSR)	Male										Female									
	IHD					n b IHD					IHD					not IHD				
	MZ (SE)	DZ (SE)	XZ (SE)	Tot M	Tot H	MZ (SE)	DZ (SE)	XZ (SE)	Tot M	Tot H	MZ (SE)	DZ (SE)	XZ (SE)	Tot M	Tot H	MZ (SE)	DZ (SE)	XZ (SE)	Tot M	Tot H
Hb (g/100 ml)	14.9 (1.4)	14.5 (0.3)	15.9 (0.3)	14.8 (0.2)	14.8 (0.2)	15.0 (0.3)	15.6 (0.3)	16.1 (0.3)	15.4 (0.3)	15.4 (0.3)	14.3 (0.4)	13.3 (0.4)	13.5 (0.4)	13.8 (0.4)	13.8 (0.4)	14.0 (0.4)	13.9 (0.4)	13.9 (0.4)	13.8 (0.4)	13.8 (0.4)
Not (%)	44.0 (1.0)	43.1 (0.9)	46.0 (0.9)	43.7 (0.6)	43.7 (0.6)	44.8 (0.8)	45.8 (0.7)	48.1 (0.7)	45.4 (0.5)	45.4 (0.5)	41.8 (0.9)	40.4 (0.9)	40.9 (0.9)	40.9 (0.7)	40.9 (0.7)	41.7 (0.6)	41.6 (0.6)	41.6 (0.6)	41.7 (0.6)	41.7 (0.6)
ZSR (mm/hour)	16.8 (5.8)	15.4 (2.9)	6.2 (1.1)	14.6 (1.3)	14.6 (1.3)	11.7 (1.9)	11.5 (1.7)	5.6 (0.7)	11.5 (1.3)	11.5 (1.3)	20.9 (5.8)	18.6 (3.4)	18.6 (3.4)	19.7 (3.4)	19.7 (3.4)	16.5 (2.4)	17.8 (2.2)	18.2 (2.2)	17.5 (2.0)	17.5 (2.0)
Total number	10	25	5	40	40	5	44	1	68	68	8	9	9	17	17	30	48	2	80	80

1) Data missing from one subject

Comments

As already mentioned the Stockholm Prospective Study has identified high hemoglobin as a factor associated with an increased risk of IHD (Böttiger & Carlson 1972). But the same study has also demonstrated a positive correlation regardless of age between the plasma lipids cholesterol and triglycerides and hemoglobin (Böttiger & Carlson 1973). Hemoglobin as a risk factor for IHD might thus be explained not by the high hemoglobin value as such but by the concomitant increase in plasma lipids (Böttiger & Carlson 1973). Furthermore the Stockholm Prospective Study has established a positive correlation between elevated ESR and cholesterol as well as triglycerides (Böttiger 1973) while a recent study on a symptomatic hyperlipidaemic person showed significantly elevated ESR compared to normolipidaemic controls (Böttiger et al 1973). The authors suggested that a possible reason for the raised ESR could be that the hyperlipidaemia causes silent vascular disease (diagnosed as ST depression during exercise) which in turn produces an elevated ESR.

3 ENVIRONMENTAL FACTORS ASSOCIATED WITH ISCHAEMIC HEART DISEASE AMONG THE DEATH DISCORDANT PAIRS

SMOKING

The relation of cigarette smoking to health has been reviewed in reports from the Royal College of Physicians in London (1971) and the Surgeon General of the U S Public Health Service (1972). A causal association probably exists between cigarette smoking and lung cancer as well as chronic obstructive lung disease. The question of a causal association with IHD has been much debated. From the studies reviewed in the above reports it is obvious that cigarette smoking is associated statistically with the excess male mortality from IHD. The connection between cigarette smoking and non-fatal myocardial infarction also seems to be strong but for angina pectoris it is more uncertain (Jenkins et al 1968; Seltzer 1968; Simborg 1970). In most studies pipe and cigar smokers have not shown any increased risk of IHD (Fletcher & Horn 1970). Although IHD death rates are much lower in women than in men, an increased mortality from IHD has been reported for women smokers too (Hammond 1966).

Both acute and chronic effects of smoking on the heart have been discussed. Among the acute effects it has been proposed that the effect of carbon monoxide or nicotine may aggravate myocardial anaemia when coronary artery flow is already reduced and it is conceivable that the risk of rhythm arrhythmias is enhanced by nicotine via the release of catecholamine (Fletcher & Horn 1970; Ross 1973). A chronic effect has been seen in the relationship between coronary atherosclerosis and cigarette smoking (Auerbach et al 1965). However Dawber & Kannel (1972) from the Framingham study consider that the cigarette habit rather than tending to accelerate atherogenesis triggers coronary attacks in persons predisposed by a sufficient degree of coronary atherosclerosis. The result of that study has shown that the risk of IHD is not associated with the duration of the habit but only with the daily intensity.

Some epidemiological evidence conflicts however with the overall picture given by the major follow-up studies. The first 3 year follow-up of nearly 13 000 men in seven countries (Key et al 1970) showed that smoking was related to later myocardial infarction and death from IHD in the U S A but not in the other countries which included East Finland with the highest male IHD mortality in the world.

One of the arguments against the findings of conventional population studies is the fact that smokers are self-selected and that other factors may be involved in the causation of IHD

It has been maintained by Friedman & Rosenman (1972) that persons with a particular behavior pattern type A characterized by aggressiveness striving and time consciousness more often develop myocardial infarction than the more placid type B; and the type A person is also more likely to be a heavy smoker

Questionnaire study on the Swedish as well as the U.S. Twin Registries disclosed an association for males between smoking and angina pectoris when the results were analysed on a non pair basis but comparisons within smoking discordant pairs showed no association with smoking for the MZ twins (report from symposium in San Juan Puerto Rico 1969) The results are supported by a clinical study on 196 smoking discordant pairs (Lundman 1966) from the Swedish Twin Registry no difference being found with regard to IHD within the smoking discordant pairs

Material: see Chapter I

Methods

Information about smoking habits was obtained from questionnaire replies received in 1967/1970. If the twins had answered in 1967 well as 1970 the latter report was used

Result

Smoking habit in the male and female death discordant pairs according to the questionnaires are shown in Tables 26-28. When present and former smoker are combined (Table 28) the percentage distribution of male smokers is somewhat but not significantly higher for the IHD compared to the not IHD death discordant pairs. Of the male twins who died from IHD 83% were smokers as against 85% of their surviving co-twins while 76% of those who died from other cause than IHD were smokers compared with 72% of their surviving co-twins. The corresponding figure for cigarette smokers in the IHD death discordant pairs are 50% and 55% compared to 40% and 52% among the not IHD death discordant pair. Nor are the differences significant

There were more former cigarette smokers (Table 26) among the deceased male twin than the surviving co-twins especially among those who died from IHD

With respect to age group (Table 28) 60% (6 of 10) of the

Table 26 Smoking habit in male d with di rdant pair n rding b earli r qu ti unaire 1 r
ntage di tribution

	MZ			DX			XX			not illd			Tot		
	D	S	Tot	D	S	Tot	D	S	Tot	D	S	Tot	D	S	Tot
Non smokers	10	70	20	16	20	0	18	15	20	16	20	16	20	16	20
Total present smokers	40	80	52	68	60	60	50	70	56	60	52	67	100	100	54
Total cigarette smokers	30	80	24	24	60	60	30	43	12	32	26	36	100	100	22
110 cigarette daily	20	30	16	1	40	20	20	18	8	28	14	21	100	0	13
all cigarettes daily	10	50	8	12	20	40	10	25	4	4	12	14	0	100	9
Cigar and/or pipe	10	0	28	44	0	0	20	28	44	28	26	31	0	0	32
Total if near smoker	50	0	28	16	0	40	33	15	24	24	21	14	0	0	22
Total cigarette smoker	30	0	16	12	20	40	20	17	24	20	14	14	0	0	18
110 cigarette daily	10	0	16	8	20	20	15	8	16	16	10	14	0	0	12
all cigarette daily	0	0	0	4	0	0	5	5	8	4	5	0	0	0	6
Cigar and/or pipe	20	0	12	4	0	0	13	3	0	4	7	0	0	0	4
Total number	10	10	25	25	5	5	40	40	5	25	42	42	1	1	68

DeDec a ed twin; S=Surviving twin

Table 27 Smoking habits in female death discordant pairs according to earlier questionnaires Per-
centag distribution

	IHD										not IHD									
	MZ					DZ					MZ					DZ				
	D	S	D	S	Tot	D	S	D	S	Tot	D	S	D	S	Tot	D	S	D	S	Tot
Non smokers	75	50	78	67	-	-	-	-	-	76	59	70	77	85	79	50	100	79	79	79
Total pre ent smoker	25	13	11	33	-	-	-	-	-	18	24	30	23	15	17	50	0	21	19	19
Total cigarette smoker	25	13	11	33	-	-	-	-	-	18	24	27	23	15	17	50	0	20	19	19
1 10 cigarettes daily	13	13	11	11	-	-	-	-	-	12	12	20	23	10	10	50	0	15	15	15
>11 cigarettes daily	13	0	0	22	-	-	-	-	-	6	12	7	0	4	6	0	0	5	4	4
Cigar and/or pipe	0	0	0	0	-	-	-	-	-	0	0	3	0	0	0	0	0	1	0	0
Total former smokers	0	38	11	0	-	-	-	-	-	6	18	0	0	0	4	0	0	0	0	3
Total cigarette smoker	0	38	11	0	-	-	-	-	-	6	18	0	0	0	4	0	0	0	0	1
1 10 cigarettes daily	0	25	11	0	-	-	-	-	-	6	12	0	0	0	2	0	0	0	0	1
>11 cigarettes daily	0	13	0	0	-	-	-	-	-	0	6	0	0	0	0	0	0	0	0	0
Cigar and/or pipe	0	0	0	0	-	-	-	-	-	0	0	0	0	0	2	0	0	0	0	1
Total number	8	8	9	9	0	0	0	0	0	17	17	30	30	48	48	2	2	80	80	80

D=Deceased twin; S=Surviving co-twin

Tabl 28 Smoking habit in male and female d with di ordant pair so ording to arli = qu ti nneir
with pr ent and former smoker ombined Pero ntag di tribution

	MZ				DZ				IHD				T t				MZ				DZ				not IHD				Tot			
	D		S		D		S		D		S		D		S		D		S		D		S		D		S					
	D		S		D		S		D		S		D		S		D		S		D		S		D		S					
MALES:																																
Men smokers																																
Total smokers																																
Total cigar tt smokers																																
110 cigar tt daily																																
111 cigarettes daily																																
Cigar and/ r pipe																																
Total number																																
FEMALES:																																
Men smoker																																
Total smokers																																
Total cigarett smoker																																
110 cigarett # daily																																
111 cigarett daily																																
Cigar and/ r pipe																																
Total number																																

DeDe seed twin; SeSurviving twin

Table 29 Distribution of death discordant male twin pairs according to concordance/discordance with respect to smoking habits (present and former smokers combined)

	Decedent twin									
	IID					not IID				
	MZ	DZ	XZ	NZ	Tot	MZ	DZ	XZ	Tot	
	NS	SM	NS	SM	NS	NS	SM	NS	SM	NS
Surviving co-twin	NS 1	SM 1	NS 0	SM 1	NS 1	NS 0	SM 1	NS 0	SM 1	NS 1
	SM 0	SM 8	NS 3	SM 18	NS 1	NS 1	SM 0	NS 7	SM 1	NS 8

Table 30 Distribution of death discordant male twin pairs according to concordance/discordance with respect to cigarette habits (present and former smokers combined)

	Decedent twin									
	IID					not IID				
	MZ	DZ	XZ	NZ	Tot	MZ	DZ	XZ	Tot	
	NS	SM	NS	SM	NS	NS	SM	NS	SM	NS
Surviving co-twin	NS 1	SM 1	NS 2	SM 1	NS 3	NS 4	SM 3	NS 0	SM 3	NS 3
	SM 0	SM 8	NS 1	SM 1	NS 2	NS 1	SM 1	NS 0	SM 1	NS 6

Table 31 Distribution of death discordant female twin pairs according to concordance/discordance with respect to smoking habits (present and former smokers combined)

	Decedent twin									
	IID					not IID				
	MZ	DZ	XZ	NZ	Tot	MZ	DZ	XZ	Tot	
	NS	SM	NS	SM	NS	NS	SM	NS	SM	NS
Surviving co-twin	NS 4	SM 0	NS 6	SM 0	NS 10	NS 19	SM 4	NS 3	SM 1	NS 55
	SM 2	SM 1	NS 2	SM 0	NS 3	NS 2	SM 5	NS 4	SM 0	NS 8

NS=Non-Smoker SM=Smoker

MZ twins who died from IHD were cigarette smokers again 80 % of their surviving co-twins. Counting only present smokers (Table 26) the corresponding figures are 30 % v 80 %. Smoking >10 cigarettes daily is significantly ($p < 0.05$) more common among the surviving male MZ twins whose partner died from IHD than among their counterparts whose partners died from other causes; this is true for present smokers (50 % v 4 %) and for present and former smokers combined (50 % v 8 %). The corresponding comparison between the deceased male MZ co-twins gives the same trend but it is not so pronounced. However with regard to the larger group of death-disorder male DZ pairs there is a higher proportion of cigarette smokers among the surviving co-twins whose partner died from other causes (50 %) compared to those whose partners died from IHD (36 %) but among their deceased partners the proportions are about the same (41 % v 40 %).

Of the female twin who died from IHD (Table 28) 24 % were smokers compared with 41 % of their surviving co-twin while the corresponding figure for the non-IHD death-disorder twins are 21 % and 21 %.

Early all the female smokers were cigarette smokers. Of the former smokers only one was found among the deceased co-twins. None of the differences recorded among the females is statistically significant.

Smoking disorders among the male death-disorder pairs is shown in Table 29-30. Only among the DZ twin pair in whom death-disorder was not caused by IHD is there an apparent smoking disorder: in 7 of the smoking-disorder pair the surviving co-twins were smokers compared to 4 pairs in which the deceased twins were smokers. If only cigarette smoking-disorder pairs are considered the corresponding figures for the non-IHD death-disorder pairs were 5 and 3. Nevertheless statistically significant differences among the females with respect to smoking disorders (Table 31).

Comments

With regard to mortality from IHD especially in men so it tends to indicate a positive relation to smoking (Doll & Hill 1964; Best 1966; Hammond 1966; Kannel 1966; Flather & Hearn 1970). The present number of smoking-disorder twin pairs among the IHD death-disorder pairs is too small to permit an evaluation of the effect of smoking on IHD mortality but it can be mentioned that in the mortality study on the whole of the Swedish Twin Registry (Friborg et al 1973) a higher mortality partly due to IHD was found among DZ smoking-disorder pairs but not among MZ pairs in both men and women. It may well be that cigarette smoking only reflects a certain constitution and that

smokers and non-smokers are self-selected groups. This view is also supported by the twin studies by Lundman (1966) and Liljefors (1970). Examining 196 twin pairs with different intra pair smoking habits, Lundman found no difference in IHD manifestations between the more and less exposed twins. In the male twin pairs studied by Liljefors, smoking habit did not differ appreciably in pairs discordant with regard to the probable presence of IHD. Neither did life time exposure, expressed as cigarette years, show any association with IHD. Nor was there any difference in smoking exposure in the infarction discordant pairs.

The finding of proportionally more cigarette smokers among the surviving male MZ co-twins whose partners died from IHD compared to those whose partners died from other causes could indicate an association between cigarette smoking and IHD. The larger male DZ group did not show similar differences in cigarette smoking habits. However, the deceased male MZ twin also displayed the same trend in cigarette smoking habit as the surviving co-twins, which may indicate that it is constitutional factors which lie behind these findings.

ALCOHOL

Studies on the association between alcohol consumption and IHD have yielded conflicting results. Chronic alcoholics have been considered to possess protection from coronary atherosclerosis. Several authors (e.g. Grant et al 1959; Stare 1961; Björck 1963) found no association between alcohol consumption and IHD. Data from the Framingham study support the opinion that alcohol is not correlated with IHD (Kannel 1966). Alcohol consumption apparently showed no association with the development of IHD, neither did it protect against atherosclerosis of the heart.

In a mortality study on approximately one million persons under the age of 40, alcoholism and heavy drinking were overrepresented among fatal IHD cases (Bainton et al 1963). A study from Norway has also shown a high mortality from IHD among chronic alcoholics compared to the general population (Sundby 1967). In a report on men born in 1913, registration at the local temperance board showed a strong association with mortality in myocardial infarction (Tibblin 1972).

Material c Chapter I

T bl J DI tributi n of 3 ath di ordant male twin pair a ording to r gi treti n in Al chot
R gi try

	IID				n t IID				Tot	
	MR	DZ	R	D	MR	DZ	R	D		
Regi t red	1	0	5	3	1	~	7	5	6	8
T t l numb	10	10	5	25	5	5	40	40	25	~5
									42	1
									42	1
									68	68

D=Dec d twin; S=Surviving co twin

Table 33 DI tributi n of 3 ath di ordant mal twin pairs according to a noordance/discordance
With re pest t registrati n in Alcohol Regi try

	IID				n t IID				Tot	
	MR	DZ	R	D	MR	DZ	R	D		
Surviving	9	1	18	4	2	1	9	6	15	~
co twin	0	0	2	1	0	4	1	1	4	4
									16	11
									7	8
									0	0
									11	12

R R giat red; MR M t R giatered

Methods

Persons with alcohol problems were defined operationally as those entered in a nationwide registry for misconduct while under the influence of alcoholic beverage. This registry has existed since 1932. All the male death discordant twins were matched with the registry.

Results

The numbers of death discordant male twins with entries in the Alcohol Registry are shown in Table 32. Entries were found for 32 out of 108 (30 %) deceased twins compared to 28 out of 108 (26 %) surviving co-twins. Of the 40 twins who had died from IHD, 7 (18 %) had been registered compared with 5 of their surviving partners (13 %), whereas of the 68 who had died from other causes than IHD, 25 (37 %) had been registered compared with 23 of their surviving partners (34 %). A distribution of the registered twins in the death discordant pairs (Table 33) shows that only one of the IHD death discordant pairs, a DZ pair, is concordant with respect to registration compared to 12 in the not-IHD group. Among the discordant (registered/not registered) pairs there are no significant differences within the death discordant pairs.

Comments

An entry in the Alcohol Registry usually indicates that the person in question has alcohol problems. It was found by Hlander (1972) that 11.5 % of male twins born 1900-1924 had at least one conviction for drunkenness compared to only 0.2 % of the female twins, the corresponding figure for male twins born 1925-1954 being 11.3 % and for females 0.6 %. Because only a few female twins have been registered, only male twins were matched with the Alcohol Registry. A mortality follow-up of 9,000 twin pairs from the Swedish Twin Registry has shown entry in the Alcohol Registry to be associated with a higher mortality regardless of smoking and it was also found that 10 % of non-smokers were registered as against 30 % of those who smoked more than 10 cigarettes a day (Friberg et al 1973). Concerning the age-specific death rate, a weak association was found between registration and IHD. In the present investigation too, registration was somewhat more common among the deceased twin irrespective of the cause of death (IHD/non-IHD). However, alcohol registration does not seem to have contributed to the high prevalence of IHD manifestation in the surviving co-twins in the IHD death discordant pairs. Using questionnaire replies in 1967 concerning

alcohol consumption Myrhed (1974) investigated 70 male twin pairs discordant with respect to alcohol consumption. The alcohol discordant pairs did not differ in IHD manifestations evaluated as ST depression in connection with exercise. A check of the questionnaire replies from 1967/1970 on alcohol consumption for the present material yielded a fairly high proportion of non respondents (27 % among the deceased male twins and 36 % among their surviving co-twins compared to 44 % among the deceased female twins and 46 % among their surviving co-twins) which is why this source of information on alcohol consumption could not be used here.

PHYSICAL INACTIVITY AND SOME SOCIAL FACTORS

Physical inactivity or sedentary living has also been implicated as a risk factor for IHD (Simberg 1970; Karvonen 1972; Stamler 1973). The findings are not a clearcut or consistent as for many other risk factors probably due to the difficulty of defining R suit from the Health Insurance Plan (Frank et al 1966) and the Framingham study (Kannel 1966) have shown a striking increase of fatal myocardial infarction among physically inactive men. A such correlation was found to angina pectoris and coronary insufficiency in the Framingham study but the Health Insurance Plan showed angina pectoris to be even more common in the more active group of men. The Westmanna collaborative study (Frank et al 1966) has also confirmed that regular exercise habit were less common among those with a fatal as opposed to a non fatal myocardial infarction. On the other hand the seven countries study (Key et al 1970) disclosed no association between the incidence of IHD and physical inactivity in any of the cohorts. It is also worth noting that the member of the cohorts from Finland had the highest incidence of IHD but were usually the most physically active and non obese.

Various social and psychosocial factors have been considered as risks for IHD (cf Jenkins 1971). The complexity of the variable and the lack of uniform methods for their investigation make such studies difficult to perform and evaluate. Stamler (1973) considered the modern way of life in highly urbanized societies to play an important role in the aetiology of IHD. Results differ with regard to the correlation of different social economic groups to IHD (Hofvindh & Helmers 1973). A higher prevalence of myocardial infarction has been noted among employers.

especially owners of small shops and sale men (Blürock et al 1958) Analyses of later studies have tended instead towards a higher prevalence and incidence of IHD in the lower socio-economic groups (Antonowsky 1968)

A low educational background has also been associated with an increased risk of myocardial infarction (Antonowsky 1968; Hinkle 1969) Social group mobility (Wardwell 1968) as well as occupational mobility (Syme 1964) are other sociologic factors which have been found to discriminate IHD patients from other subject

According to Friedman & Rosenman (1959) individuals exhibiting an emotional complex characterized by an excessive sense of time urgency drive and competitiveness (type A behavior) are more prone to the onset of IHD than are individuals of the converse type of behavior (type B) Type A subjects usually have elevated cholesterol values and are also more often heavy smokers than type B subject Other psychosocial characteristics found in men with myocardial infarction seem to be job dissatisfaction (Sales & House 1971; Theorell 1971) and overtime work (Kasanen et al 1963; Liljefors 1970)

Material: see Chapter I

Method

Information on physical inactivity and some social factors (change of place of work extra work and place of residence) was obtained from questionnaires mailed to all twins in 1967 and 1970 while information on education was obtained from questionnaires in 1962 The twins were asked among other things if they had changed their place of work after the age of 25 and how often if they had had extra work beside their ordinary job and if this happened periodically They were also asked about their place of residence after the age of 25 and if this had been mostly in big cities Higher education is taken here to be education above the compulsory school including folk-high school vocational school and evening courses The twins were also asked about how much exercise they had taken when 25-50 years of age; the answer "hardly any" was recorded here

Results

The distribution of death discordant pairs according to their answers on physical inactivity and various sociologic variables is given in Tables 34 and 35 Hardly any increase when 25-50 years of age is

Table 35 Physical inactivity and socioeconomic variables in the female death discordant pairs according to earlier questionnaires. Absolute numbers and prevalence rates in % (parentheses)

	MZ				DZ				XZ				Tot				not DZ				Tot	
	D		S		D		S		D		S		D		S		D		S		D	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Hardly any exercise	2	0	1	0	3	0	1	5	7	0	0	7	8	0	0	7	8	0	0	7	8	
	(25)		(11)		(18)		(7)	(3)	(10)	(15)			(9)	(10)								
Change of place of work > 5 times	1	0	0	0	1	0	1	2	4	0	0	3	4	0	0	3	4	0	0	3	4	
	(13)				(6)		(7)	(2)	(4)			(4)	(5)									
Extra work	4	0	1	3	3	3	5	8	15	16	0	20	4	0	0	20	4	0	0	20	4	
	(25)		(11)	(33)	(18)	(18)	(17)	(27)	(31)	(33)		(25)	(30)									
Place of residence • Mostly big cities	0	0	0	0	0	0	7	4	9	10	0	15	1	0	0	15	1	0	0	15	1	
							(23)	(13)	(19)	(21)		(19)	(19)									
Higher education	2	2	2	1	4	3	7	7	10	10	0	1	18	0	0	1	18	0	0	1	18	
	(25)	(5)	(22)	(11)	(24)	(18)	(23)	(23)	(21)	(21)		(50)	(23)									
Total number	8	8	9	9	17	17	30	30	48	48	2	80	80	2	2	80	80	2	2	80	80	

D=Deceased twin; S=Surviving co-twin

reported slightly more often among the IHD death discordant male pairs than among the not IHD death discordant pairs (0% vs 18% compared to 10% vs 10%). Among the IHD death discordant female twin pair 3 (18%) of the deceased twins had reported hardly any exercise vs none of the surviving co-twins. With regard to change of place of work extra work and place of residence there are no substantial differences between the surviving and the deceased twin in the IHD death discordant male and female pairs. None of the female twins in the IHD death discordant pairs had mostly lived in big cities whereas this applied to 19% in the not IHD group. With regard to higher education (above compulsory school) the proportion of educated subjects is much the same in the IHD and not-IHD male death discordant pairs but there are more marked differences within the various zygosity groups. Among the female IHD death discordant pair the proportion of twin with higher education is 24% vs 18% compared to 1% vs 23% in the not IHD group. None of the differences is statistically significant.

Comment

The sociologic data in the present study concern factors which may possibly predispose for IHD. However precipitating factors such as various life changes have been shown to precede the onset of myocardial infarction (Thorell 1971) and sudden death (Rah & Lind 1971) but the accumulation of life change prior to the onset of disease does not seem to be a specific sign for IHD (Rah 1969).

To judge from the result of this study it does not seem very likely that the social factors recorded could be responsible for the increased prevalence of IHD manifestations in the surviving co-twin in the IHD death discordant pair. In the twin study by Liljefors (1970) ambition and overtime work were noted significantly more often among the affected partner in the infarction discordant MZ pair.

It should be born in mind however that in retrospective studies the diseased subject often tends to be more aware of signs and symptoms associated with the disease in question.

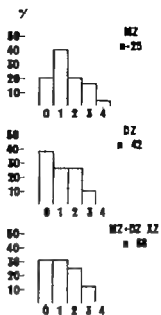
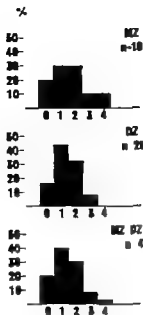
Table 38 Respondent co twins tabulated according to the cumulative distribution of certain defined biometric risk factors

Number of risk factors	MALES										FEMALES									
	IHD					not IHD					IHD					not IHD				
	MZ No (%)	XZ No (%)	Tot No (%)	MZ No (%)	DX No (%)	XZ No (%)	Tot No (%)	MZ No (%)	DX No (%)	XZ No (%)	Tot No (%)	MZ No (%)	DX No (%)	XZ No (%)	Tot No (%)	MZ No (%)	DX No (%)	XZ No (%)	Tot No (%)	
≥ 1	8 (80)	21 (84)	3 (60)	32 (80)	20 (80)	26 (62)	1 (100)	47 (69)	8 (100)	8 (89)	-	16 (94)	76 (87)	40 (83)	2 (100)	68 (85)				
≥ 2	5 (50)	10 (40)	1 (20)	16 (40)	10 (40)	15 (36)	1 (100)	26 (38)	7 (88)	7 (78)	-	14 (82)	18 (60)	25 (52)	2 (100)	45 (56)				
≥ 3	2 (20)	2 (8)	0 (0)	4 (10)	5 (20)	4 (10)	0 (0)	9 (13)	4 (50)	3 (33)	-	7 (41)	9 (30)	11 (23)	2 (100)	22 (28)				
≥ 4	1 (10)	0 (0)	0 (0)	1 (3)	1 (4)	0 (0)	1 (1)		0 (0)	0 (0)	-	0 (0)	0 (0)	1 (2)	1 (1)					
≥ 5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	-	0 (0)	0 (0)	0 (0)	0 (0)					
Number of co twins	10	25	5	40	25	42	1	68	8	9	-	17	30	48	2	80				

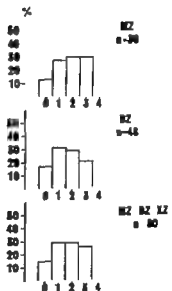
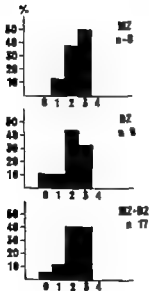
Table 39 = Pond net o twin absolute and o rd mg o = environmental risk factors

Numb of risk factors	MALES										FEMALES									
	IID					not IID					IID					not IID				
	ME	ME	XE	Tot	ME	ME	XE	Tot	ME	ME	ME	XE	Tot	ME	ME	ME	XE	Tot	ME	ME
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
≥ 1	10	25	4	39	25	39	1	65	8	9	8	9	17	26	46	2	74			
	(100)	(100)	(80)	(98)	(100)	(95)	(100)	(96)	(88)	(89)	(100)	(100)	(100)	(87)	(96)	(100)	(93)			
≥ 2	8	18	3	29	22	28	1	51	7	8	7	8	15	23	32	2	57			
	(80)	(77)	(60)	(73)	(88)	(67)	(100)	(75)	(88)	(89)	(88)	(88)	(88)	(77)	(67)	(100)	(71)			
≥ 3	4	11	0	15	10	14	1	5	4	5	4	5	9	15	20	2	37			
	(40)	(44)		(38)	(40)	(33)	(100)	(37)	(50)	(56)	(50)	(56)	(53)	(50)	(42)	(100)	(46)			
≥ 4	3	3	0	6	4	5	0	9	1	2	1	2	3	4	8	0	12			
	(30)	(12)		(15)	(16)	(12)		(13)	(13)	(22)	(13)	(22)	(18)	(13)	(17)		(15)			
≥ 5	2	1	0	3	1	1	0	2	0	0	0	0	0	0	0	1	0			
	(20)	(4)		(8)	(4)	(2)		(3)								(3)	(3)			
≥ 6	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0			
	(10)			(3)																
≥ 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Numb of o-twins	10	25	5	40	25	42	1	68	8	9	17	30	48	2	80					

Males



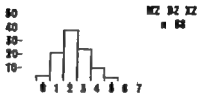
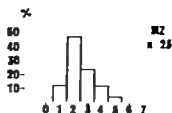
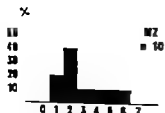
Females



NUMBER OF RISK FACTORS

Fig 8 Distribution of respondent co-twins according to certain biometrical risk factor. Black column indicates that the partner died from IHD and white columns that the partner died from other causes.

Males



Females



NUMBER OF RISK FACTORS

Fig 9 Distribution of concordant co-twins according to certain defined biometric and environmental risk factor. Black column indicate that the partner died from IHD and white column that the partner died from the cause.

None of the differences with regard to the number of risk factors is statistically significant

Comments

Before combining risk factors it is necessary to define risk levels for each factor. The cut-off points used here have been chosen fairly liberally which naturally increases the sensitivity but decreases the specificity with regard to the risk of IHD. It seems however that the risk rises steadily from the lowest to the highest values for most of the continuous risk factors (Dawber & Kannel 1972; Stamler & Epstein 1972).

New statistical methods for multivariate analyses have been applied more recently to evaluate the risk of IHD when several risk characteristics are considered simultaneously. In the Framingham study a multiple logistic model was used to compute risk probabilities for each individual (Truett et al 1967). Coefficients for each of the risk factors were based on the risk factor level at the first examination and the 12 year IHD incidence.

The relative importance of risk factors is however not the same in all populations. Furthermore the multivariate risk functions have not yet been translated into clinical terms. A combination of risk factors defined by arbitrarily chosen levels can therefore be useful as a broad classification of high risk individuals.

In the present study the number of risk factors does not differ significantly between co-twins whose partners died from IHD and those whose partners died from other causes. When only biometric risk factors are combined the same tendencies are found for both males and females with more risk factors among the MZ than the DZ co-twin irrespective of the cause of death of the partner (IHD/not IHD). Furthermore the co-twins with partner who died from IHD have somewhat more risk factors than those whose partners died from other causes, the difference being more pronounced among the females.

Twin studies cited in connection with the separate risk factors have shown that the biometric risk factors are governed to varying degrees by heredity but may also be influenced by the environment. In a prospective study of 50-year old men in Gothenburg the occurrence of risk factors had a predictive value not only for the incidence of myocardial infarction but also for early death from other causes (Tibblin 1972). The difference in number of risk factors between MZ and DZ co-twin irrespective of the cause of death of the partner might thus reflect constitutional differences. The male co-twins with partner

who died from IHD have rather more biological risk factors than those whose partners died from other causes but this difference is not very marked possibly because the so called risk factors are not as specific for IHD as used to be thought. According to Tibblin (1972) some common factor such as premature aging could be responsible for an increased number of risk factors as well as the development of myocardial infarction and death from non-cardiovascular cause.

The difference in the number of biometrical risk factors show the same trends when environmental risk factor (cigarette smoking, physical inactivity and extra work) are included in the possible combination. The environmental factor however contributed proportionally less to the increased number of factors among females especially the MZ co-twin whose partner died from IHD than among the males. Harvald & Hauge (1968) in their study on Danish twin pairs found that the occurrence of fatal coronary occlusion to be genetically determined to a much larger extent in females than males. They considered environmental factors to play a greater part in the etiology of IHD in males compared to females.

In a comparison between male and female populations in Gothenburg with respect to risk factors for IHD (Bengtsson et al 1973) it was concluded that sex difference in the factor apparently explain only part of the difference in incidence of myocardial infarction in men and women. Smoking, alcohol consumption and triglycerides were however more common among the male.

The present difference in the number of risk factors do not significantly discriminate the surviving co-twins with partner who died from IHD and those whose partner died from other reasons which partly could be due to a lack of specificity in the risk factor. The tendencies however are consistent with a somewhat higher number of risk factors among the co-twin whose partner died from IHD. The slight differences in the number of risk factor may reflect different constellations but it is all probable that genetic factor operated predominantly of the established risk factor in the development of IHD. Firmer conclusion in this respect will have to await a mortality follow up of the examined twins.

IV
GENERAL DISCUSSION

Epidemiological studies have indicated that IHD has a multifactorial etiology. From the standpoint of preventive action most interest has been focused on the environmental factors which can be modified and controlled and there have been relatively few studies on the importance of familial factors and the genetic predisposition for IHD which seem to be especially important for detecting individuals at high risk. Twin studies probably offer the best tool with which to study the relative influence of genetic and environmental factors.

Various theoretical models in twin studies were discussed at an international twin symposium in Puerto Rico in 1969 but it was also pointed out that there is no generally accepted methodology in the design of twin studies and that the possibilities are many and complex. It was suggested that clinical examination of the partners of twins who have died could be considered as a valuable extension of mortality studies.

One of the main purposes of the present study is to evaluate a genetic influence in IHD - by examining whether the occurrence of IHD in the surviving co-twin is associated with the partner's cause of death (IHD/not IHD).

The present material of death discordant pairs comprises 78.2 % of a sample selected from the Swedish Twin Registry. This response rate must be considered fairly high partly because the population drawn from the whole of Sweden and partly in view of the fact that the co-twins had lost their partner on average 5 months earlier.

The accuracy of cause of death determinations depends mainly on a high autopsy rate. Because the autopsy rate for the deceased twins in the present material was about 57 % other sources of information (hospital records etc.) were consulted in order to make the mortality evaluation as reliable as possible.

This was especially important because the IHD material included cases of sudden death (ISC number 795/99). If there was a strong suspicion that a cause of sudden death had some other explanation than IHD as the underlying cause then it was not included in the IHD group. About 70 % of the sudden death included as IHD cause had either a preexisting history of IHD (myocardial infarction and/or angina pectoris on effort) or findings at autopsy indicating IHD (signs of fresh or old infarct and/or severe atherosclerotic change of the coronary arteries) as compared to about 4 % of the twin who died from other cause than

IHD. The other 30 % of the IHD sudden deaths had no such earlier evidence of this disorder; they were included because there was no reasonable explanation for the death. This is in line with other studies in which the initial clinical manifestation of IHD was found to be sudden death in about 20-25 % of IHD cases (Kuller et al 1966; Kuller 1966). In the present investigation analysis of the occurrence of IHD and risk factors among the surviving co-twins have been related to the cause of death of the partner (IHD not IHD). In addition to the case of sudden death the IHD death discordant pairs thus included those where the cause of death was myocardial infarction. Pooling these two diagnoses has been done in many epidemiological studies seems to be justifiable not only because about 70 % of the sudden deaths showed evidence of previous IHD but also because the risk factors for the sudden death cases are in general agreement with those for myocardial infarction (Vedin et al 1973). Neither did the relatively small number of IHD death discordant pairs permit a further breakdown into subgroup.

The occurrence of IHD in the surviving co-twins was established by determining the prevalence of angina pectoris, myocardial infarction and ECG change suggestive of IHD. Angina pectoris and myocardial infarction were found more commonly among the surviving co-twins whose partners died from IHD than among those whose partners died from other causes. Among females this was true for angina pectoris. The trend was clear with regard to myocardial infarction among males but none of the differences is statistically significant. The relatively low prevalence of manifest IHD in conjunction with the small number of MZ and DZ co-twins makes it difficult to assess the significance of heredity. According to Epstein in (1964) a prevalence rate of 5.5 % for manifest IHD represents only the top of the iceberg. It has been considered that ST depression in connection with exercise could be a fairly sensitive and specific predictor of coronary obstructive disease (Helfant et al 1973). The appearance of ST segmental depressions in response to exercise has been shown to be a powerful predictor of overt IHD (Mattingly 1962; Astrand & Lundman 1968; Blackburn et al 1970; Doyl & Kinch 1970). Using ST depression in connection with exercise as a sign of IHD among the surviving co-twins the frequency among both the male and the female co-twins whose partners died from IHD was significantly higher than among the co-twins whose partner died from other causes. In the males this difference was significant for ST depression ≥ 1 mm but in the females only when ST depressions of >0.5 mm were included. The difference certainly reflects constitutional difference. The impression of a substantial genetic influence in IHD is strengthened

smokers especially ≥ 11 cigarettes daily proportionally more often than the other male MZ and XZ twins a circumstance which may have contributed to the corresponding difference in IHD manifestations. However the deceased MZ and XZ twins also displayed the same tendencies in the smoking of cigarettes. Thus it is possible that the differences with regard to cigarette smoking merely reflect differences in constitution which could play the major part also with regard to the development of IHD.

Among the men born in 1913 (Tibblin 1972) it seemed that being registered with the local temperance board was strongly associated with myocardial infarction. All the male death discordant twins in the present investigation have been matched against a register for misconduct involving alcohol. Registration was somewhat more common, although not significantly so among the deceased twins irrespective of the cause of death (IHD/not IHD).

The questionnaire data used in this study on sociologic background variables represent factors which could predispose for IHD. These findings however do not preclude the operation of precipitating factors such as various life changes which have been shown to precede the onset of myocardial infarction (Theor 11 1971) and sudden death (Rahe & Lind 1971).

The high risk of developing IHD has been demonstrated among individuals with multiple risk factors (Dawber et al 1957; Kannel et al 1961; Kannel et al 1967; Tibblin & Wilhelmsson 1971; Böttiger & Carlson 1972; Stamler & Epstein 1972). The risk factor used in the combination were defined with fairly liberal cut off points which is a practical and common way of defining risk level. Risk evaluation by means of new statistical methods for multivariate analysis seems to be a more physiological approach considering that the risk steadily increases from the lowest to the highest value for most of the continuous variable (Dawber & Kannel 1972; Stamler & Epstein 1972). These methods have not yet been translated into clinical terms and the coefficients for each of the risk factors are based upon special populations. Using a multivariate risk function (Wilhelmsen et al 1973) based on the variables cholesterol, systolic blood pressure and smoking it has been shown (Elmfeldt 1974) that a fairly high proportion of men developed myocardial infarction notwithstanding their classification as low risks.

When only biometric risk factors were combined in the present study more risk factors were noted among the MZ co-twins, DZ co-twins irrespective of the cause of death (IHD/not IHD). The same trend was found when the environmental risk factors (

smoking physical inactivity and extra work) were included in the possible combination. The environmental factors contributed proportionally less to the increase in the number of risk factors among the female especially the MZ co-twins whose partner died from IHD than among the males. Environmental factors have been found to play a less important role in the development of IHD in women (Harvald & Hauge 1968; Bengtsson 1973). The differences found in the number of risk factors did not significantly discriminate the surviving co-twins whose partner died from IHD from those whose partner died from other reasons a circumstance which could be due to an insufficient specificity of the risk factor. Incidence data from the prospective study on men born in 1913 (Tibblin 1972) have shown that most of the risk factors which have been considered to be rather specific for the subsequent occurrence of myocardial infarction also appear to be also valid for early death from whatever cause.

To judge from the result of the present study it can be concluded that a considerable genetic influence seems to operate in the development of IHD. Genetic influences are transmitted through one of the established risk factors but it is possible that unknown risk factors or a general biologic factor as premature ageing (Tibblin 1972) or low life potential (Riörok 1974) constitute the most important underlying factor in the development of IHD. Dawber & Kannel (1972) have proposed that the continued high morbidity and mortality from IHD could be a consequence of ageing and genetic make-up and that the important factor contributing to the development of this disease have not been identified satisfactorily. It has also been proposed by Epstein (1964) that if one could identify and measure all of the predisposing traits as underlying biologic disturbance in terms of metabolism or other defects then it would probably emerge that they are more common than the prevalence of the disease would suggest and show more clear-cut distributions among family members. It was furthermore concluded that the prevention of IHD demands that the carriers of this trait be identified so that prophylactic measures can be instituted at an early age among genetically susceptible individuals.

GENERAL SUMMARY AND CONCLUSIONS

All the twins in the Swedish Twin Registry have been the subject of a continuous mortality follow-up ever since the Registry was established in 1959-1961. The complete twin registry is matched regularly against a total death registry for Sweden at the Central Bureau of Statistics and this procedure makes it possible to obtain the death certificates. Since 1971 the matching has been done every month. This procedure was a prerequisite for the present study.

The principal object of this investigation was to evaluate the genetic influence in IHD in the examined co-twins and to create a basis for their further mortality follow-up thereby also paving the way for assessments of the predictive value of the measured risk factors and the hereditary influence.

All unbroken male and female twin pairs from the Swedish Twin Registry below the age of 70 who in the period January 1st 1971 to March 15th 1973 became death discordant were selected for the study. 78.2 % or 205 of the surviving co-twins could be examined on average about 5 months after the death of the partner.

The cause of death of the partner was established by a team of physicians on the basis of all the assembled information (hospital records, autopsy protocols etc.). The cause of death was classified according to the International Statistical Classification of Diseases.

The zygosity diagnoses were taken from the record in the Twin Registry which is based upon questions as to similarity. If the twins in a pair had given conflicting answer they were classified as unknown zygosity (XZ).

Among 108 male death discordant pairs the cause of death was IHD in 40 pairs (10 MZ, 25 DZ and 5 XZ) and other than IHD in 68 pairs (25 MZ, 42 DZ and 1 XZ). Among 97 female death discordant pairs the cause of death was IHD in 17 pairs (8 MZ, 9 DZ) and other than IHD in 80 pairs (30 MZ, 48 DZ and 2 XZ).

191 of the surviving co-twins were examined at the Serafimer Hospital and the other 14 at their local hospital.

A sociologic and medical history was taken using questionnaires. The diagnosis of angina pectoris was established by interview according to the questionnaire designed by the London School of Hygiene and Tropical Medicine. Myocardial infarction was considered established when it had been verified at hospital. Blood pressure determinations, anthropometric measurement and X-ray of the heart and lungs were

performed as well as ECG before during and after an ergometer test. Blood samples were drawn after an overnight fast for analysis of cholesterol triglycerides uric acid and blood sugar. Erythrocyte sedimentation rate hemoglobin and hematocrit were also determined. The urine was tested for proteinuria and glucosuria.

Myocardial infarction had occurred in 15% of the surviving male co-twins whose partners had died from IHD (3 or 30% of the MZ co-twins and 3 or 12% of the DZ co-twins) as compared to only 3% of the surviving co-twins whose partners had died from other causes than IHD. Only one of the female co-twins had had myocardial infarction. If angina pectoris, pathologic Q-wave and significant ST depression ≥ 0.5 mm in connection with exercise were also included in the criteria of IHD, both male and female co-twins (pooled zygosity groups) whose partners died from IHD displayed the signs significantly ($p < 0.05$) more often than those whose partners died from other causes. In the males but not in the females the difference was significant even when ST depressions ≥ 1.0 mm were included in the criteria of IHD. Similarly, the male MZ co-twins whose partners died from IHD displayed significantly more IHD manifestations ($p < 0.05$ with ST depressions ≥ 1.0 mm and $p < 0.01$ with ST depressions ≥ 0.5 mm) than the MZ co-twins whose partners had died from other causes. Excluding ST depressions coded in the presence of high R wave amplitude (Minnesota code 31) which are thought to reflect a left ventricular hypertrophy due to hypertension, the differences in the females are no longer significant.

Most of the biometric factors measured (relative weight, skinfold thickness, blood pressure, lipid, uric acid) showed somewhat higher values for the co-twins whose partners died from IHD compared to those whose partners died from other causes. The difference in causal systolic blood pressure was significant ($p < 0.05$) among the female. Overt diabetes mellitus showed about the same prevalence in the discordant group (IHD not IHD). Nor were significant differences found with regard to hematocrit, hemoglobin or erythrocyte sedimentation rate which our authors have designated as risk factors for IHD. Information obtained earlier through mailed questionnaires about smoking habit, physical inactivity, extra work, change of place of work, education and place of residence did not significantly discriminate the deceased and the surviving co-twins. The IHD discordant pairs had a somewhat higher proportion of smokers (83% vs 85%) compared to the not IHD (76% vs 72%). To smoke ≥ 11 cigarettes daily was significantly ($p < 0.05$) more common among the surviving male MZ co-twins whose partners died from IHD than among those whose partners died from other causes. The corresponding comparison between

the deceased male MZ co twins gave the same trend but not so pronounced

All male death discordant twins were matched against a nationwide register for misconduct while under the influence of alcohol. Registration was more common among the not-IHD death discordant pairs. The deceased partners had been registered somewhat more often irrespective of the cause of death (IHD not IHD).

The biometric risk factors (relative weight ≥ 110 according to a height weight index, basal systolic blood pressure ≥ 160 mm Hg and/or basal diastolic blood pressure ≥ 95 mm Hg, cholesterol ≥ 250 mg/100 ml and/or triglycerides ≥ 150 mg/100 ml and overt diabetes mellitus) were then combined with the environmental risk factors (cigarette smoking, extra work and physical inactivity). It was found that the surviving co twins whose partners died from IHD had on average a somewhat higher number of biometric risk factors than those whose partners died from other cause, the difference being more pronounced among the female co twins. The inclusion of environmental risk factors contributed proportionally less to the number of risk factors among the females, especially the MZ co twins.

The conclusions from the present investigation could be summarized as follows:

- 1) The present results indicate a substantial genetic influence in the development of IHD.
- 2) In males the risk factors measured singly and in combination seem to explain only part of the difference in IHD manifestations between the surviving co twins whose partners died from IHD and those whose partners died from other causes. In females however elevated blood pressure may explain a great deal of the difference found in IHD manifestations. The genetic influences are probably transmitted not only through one of the risk factors measured but also through factors which are still unknown.
- 3) The environmental factors recorded by earlier questionnaires did not discriminate significantly between the deceased and the surviving co twin.

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1. ADLSON, L. Sudden death from coronary disease: the cardiac conduction. *Postgrad. Med. J.* 30:127 1955
2. ALLEN, G. HAAVALD, B. & SHIELDS, J. Measures of twin concordance. *Acta Genet. (Basel)* 17:475 1967
3. ANTONOWICZ, A. Social class and the major cardiovascular diseases. *J. Chronic Dis.* 21: 63 1968.
4. ARI, A. Serum lipids and lipoproteins in first degree relatives of young survivors of myocardial infarction. *Acta Med. Scand. suppl.* 652, 1973.
5. ATHEROSCLEROSIS STUDY GROUP: Primary prevention of the atherosclerotic diseases. *Circulation* 47: suppl. 3 1970
6. AUERBACH, O. HAMMOND E.C. & GARTINKEL, L. Smoking in relation to atherosclerosis of the coronary arteries. *N. Engl. J. Med.* 273:778, 1965
7. BAINTON, C.R. & PETERSON D.R. Death from coronary heart disease in persons fifty years of age and younger. A community-wide study. *N. Engl. J. Med.* 268:569 1963.
8. BAUMONT, J.L. CARLSON, L.A. COOPER, G.R. FEYER, Z. FREDERICKSON, D.S. & STRASSER, T. Classification of hyperlipidaemias and hyperlipoproteinaemias. *Bull. WHO* 43, 1970 p. 81
9. BORDICK, R.R. Coronary heart disease in identical female twins. *Am. J. Med.* 34:234 1963.
10. BRANTZON, C. Ischaemic heart disease in women. A study based on a randomized population sample of men and women with myocardial infarction in Göteborg, Sweden. *Acta Med. Scand. suppl.* 649 1973.
11. BROCHTOLD P. KRÖNTORP P. GUSTAFSON, A. LINDHOLM, B. TIERLIN, B. & WILHELMSSON L. Glucose tolerance, plasma insulin and lipids in relation to adipose tissue cellularity in men after myocardial infarction. *Acta Med. Scand.* 191:35, 1972
12. BRUASCONI, P. BROCHIER, M. & ZERAFTA, P. Angine de poitrine chez deux jeunes. *Arch. Mal. Coeur* 60:174, 1967
13. BRY, E.W.R. A Canadian study of smoking and health. Department of National Health and Welfare, Ottawa, 1968.
14. RÖRCK, G. Hypokaemia vero in coronary disease. *Br. Heart J.* 3:17 1964.
15. RÖRCK, G. HLOMQUIST G & SIEVERS, J. Cholesterol values in persons with myocardial infarction and in normal control group. *Acta Med. Scand.* 184:193, 1967
16. RÖRCK, G. HLOMQUIST G & SIEVERS, J. Studies on myocardial infarction in Malmö 1936-1964 II. Infarction rate by occupational group. *Acta Med. Scand.* 181:1, 1968.
17. RÖRCK, G. Environment and disorders of the circulatory system. *Scot. M.T. Acad. Med.* 35:3, 1969
18. RÖRCK, G. Factors of risk in coronary artery disease. *Mal. Carbov.* 4:11, 1963.
19. RÖRCK, G. & NYLÉN, G. Comparative trends in four Scandinavian Counties of mortality from atherosclerotic and degenerative heart disease during the years 1973-1960. *Acta Med. Scand.* 177:162, 1965
20. RÖRCK, G. HOLMSTEDT, A.M. & GYLLENHALLMAR, K. Symptomatik på ökad föett i atherosclerotisk hjärtsjukdom i Sverige. *Läkarsällningen* 67:9993, 1970
21. RÖRCK, G. & WIKLAND, R. Sudden death: what are we talking about. *Circulation* 46:236, 1972.
22. RÖRCK, G. Ideas and Concepts in the Prevention of Ischaemic Heart Disease. To be published in *Reviews of Epidemiology and Social Medicine*, 1974
23. RUTULF P. Atherosclerosis and body-build. *Acta Med. Scand. suppl.* 349, 1969
24. RUTULF P. GARLUND, T. & STERBOFF M.H. On epidemiologic methods for recording ischaemic heart disease. *Acta Med. Scand. suppl.* 474, 1967
25. KRÖNTORP P. & KRÖSTHOLM, L. Number and size of adipose tissue fat cells in relation to triglycerides in human obesity. *Metabolism* 20:703, 1971.
26. BLACKBURN, H. KEYS, A. SIMMONSON, K. BAUTAHANU, P. & PUNJARI, S. The electrocardiogram in population studies. A classification system. *Circulation* 41:160, 1960.
27. BLACKBURN, H. TAYLOR, H.L. & KEYS, A. Coronary heart disease in seven countries. XVI. The electrocardiogram in prediction of five-year coronary heart disease incidence among men aged forty through fifty-five. *Circulation* 41, suppl. 1, 154, 1970.
28. HLOMQUIST G & RÖRCK, G. Coronary mortality in relation to total mortality. *Acta Med. Scand.* 179:339 1965
29. HLOMQUIST G. The Frank lead exercise electrocardiogram. *Acta Med. Scand. suppl.* 440 1963.
30. HLOMQUIST G. Use of exercise testing for diagnosis and functional evaluation of patients with atherosclerotic heart disease. *Circulation* 44:1128 1971.

31. BOLANDER, A. M. Cause of death statistics - a tool in medical research and especially in epidemiological survey. Solicited paper to the 1973 General Conference of the International Union for the Scientific Study of Population. Statistiska Centralbyrån, Stockholm, 1973.
32. BERTON, M. I. Diagnostic errors discovered at autopsy. *Acta Med. Scand.* 1974. In Press.
33. BÖTTIGER, L. E. & CARLSON, L. A. Stockholm Prospective study II - also *Ärs sjukvårdning, Läkartidningen* 80: 4040 1975.
34. BÖTTIGER, L. E. & CARLSON, L. A. Hemoglobin, plasma lipids, and coronary heart disease. *Am. Heart J.* 86:442 1973.
35. BÖTTIGER, L. E., CARLSON, L. A., EKLUND, L.-G. & OLSSON, A. G. : Raised erythrocyte sedimentation rate in asymptomatic hyperlipidaemia. *Brit. Med. J.* 2 681 1972.
36. BÖTTIGER, L. E. Erythrocyte sedimentation rate and plasma lipids. *Acta Med. Scand.* 193:83, 1973.
37. CARLSON, L. A. Serum lipids in normal men. *Acta Med. Scand.* 167:377 1960.
38. CARLSON, L. A. Serum lipids in men with myocardial infarction. *Acta Med. Scand.* 167:399 1960.
39. CARLSON, L. A. & BÖTTIGER, L. E. Ischaemic heart-disease in relation to fasting values of plasma triglycerides and cholesterol. Stockholm Prospective Study. *Lancet* 1 668 1972.
40. CEDERLÖF, R., FRIBERG, L., JONSSON, E. & KAIJ, L. Studies on similarity diagnosis in twins with the aid of mailed questionnaires. *Acta Genet.* 11:338, 1961.
41. CEDERLÖF, R. The Twin Method in Epidemiological Studies on Chronic Diseases. Karolinska Institute, 1968 a.
42. CEDERLÖF, R., JONSSON, E. & KAIJ, L. Respiratory symptoms and angina pectoris" in twins with reference to smoking habits. An epidemiological study with mailed questionnaire. *Arch. Environ. Health* 13:728, 1968 b.
43. CEDERLÖF, R. Urban factors and prevalence of respiratory symptoms and angina pectoris" A study on 8,100 twin-pairs with the aid of mailed questionnaires. *Arch. Environ. Health* 13:743, 1968 c.
44. CEDERLÖF, R., JONSSON, E. & LUKUMÄN, T. On the validity of mailed questionnaires in diagnosing angina pectoris and "bronchitis". *Arch. Environ. Health* 13:738, 1968 d.
45. CEDERLÖF, R., FRIBERG, L. & JONSSON, E. Hereditary factors and angina pectoris. A study on 5,877 twin-pairs with the aid of mailed questionnaires. *Arch. Environ. Health* 14:397 1967 a.
46. CEDERLÖF, R., EDFOES, M. I., FRIBERG, L. & JONSSON, E. Hereditary factors, spontaneous cough and smoken cough. A study on 7 800 twin-pairs with the aid of mailed questionnaires. *Arch. Environ. Health* 14:401 1967 b.
47. CEDERLÖF, R., FLODÉN, S. & FRIBERG, L. Cancer in MZ and DZ twins. *Acta Genet. Med. Gemellol.* 18 63, 1970.
48. CERASI, E. & LART, R. Insulin response to glucose infusion in diabetic and non-diabetic monozygotic twin pairs. Genetic control of insulin response? *Acta Endocrinol.* 66:830, 1967.
49. CHIANG, H. N., PERLMAN, L. V., OSTRANDER, L. D. JR. & EPSTEIN, F. H. Relationship of premature atherosclerosis to coronary heart disease and sudden death in the Tecumseh epidemiologic study. *Ann. Intern. Med.* 70:1168, 1969.
50. CHIANG, H. N., PERLMAN, L. V., FULTON, M., OSTRANDER, L. D. JR. & EPSTEIN, F. H. Predisposing factors in sudden cardiac death in Tecumseh, Michigan. A prospective study. *Circulation* 41:31, 1970.
51. COSE, S., KING, E. & CHEN, E. Differences between respondents and nonrespondents in a morbidity survey involving clinical examination. I. Chronic Dis. 6:86 1967.
52. CRAMÉR, K., PAULIN, S. & WERKÖ, L. Coronary angiographic findings in correlation with age, body weight, blood pressure, serum lipids and smoking habits. *Circulation* 33:888, 1966.
53. DAHLBÖG, G. Twin births and twins from hereditary point of view. Stockholm, 1924.
54. DAWBER, T. R., MOORE, F. E. & MANN, G. V. II. Coronary heart disease in the Framingham study. *Am. J. Public Health* 47:4, 1957.
55. DAWBER, T. R. & KANNEL, W. B. Current status of coronary prevention. Lessons from the Framingham study. *Prev. Med.* 1:499 1972.
56. DEUTSCHER, S., OSTRANDER, L. D. & EPSTEIN, F. H. Familial factors in premature coronary heart disease - preliminary report from the Tecumseh Community Health Study. *Am. J. Epidemiol.* 81:222, 1970.
57. DOAN, A. E., PETERSON, D. R., BLACKMON, J. R. & BRUCE, R. A. Myocardial ischemia after maximal exercise in healthy men. A method for detecting potential coronary heart disease. *Am. Heart J.* 68:1 1965.
58. DOLL, R. & HILL, A. B. Mortality in relation to smoking: Ten years' observation of British doctors. *Br. Med. J.* 1:1245, 1964.

19. DOUGLAS, A.H. Ischemic heart disease in monozygotic twins. *Dis. Chest*, 49:122 1965
20. DOWNEY, W.W. DUTLE, J.A. GREGG W.R. BUCHANAN, W.W. & ALEPA, F.H. Relative roles of genetic and environmental factors in control of blood pressure in normotensive subjects. *Br. Heart J* 31:11, 1969.
21. DUTLE, J.T. & KINCH, R.H. The prognosis of an abnormal electrocardiographic stress test. *Circulation* 41: 644, 1970.
22. EDWARDS, D.A.W. HANSON, W.H. HEALY, M.J.R. TAYLOR, J.M. & WHITEHOUSE, R.H. Design and accuracy of calipers for measuring subcutaneous tissue thickness. *Br. J. Nutr.* 9:123, 1958
23. EDWARDS, J.H. The genetic basis of common disease. *Am. J. Med.* 34:227 1963
24. EKLÖF, D. Hjärtinfarkt i Göteborg 1963-1970 Morbiditet och mortalitet. Karaktäristika för överlevande och i sjukhuset med den vanliga befolkningen. Erläsnings Boktryckeri, Göteborg. 1974
25. EPSTEIN, F.H. Hereditary aspects of coronary heart disease. *Am. Heart J.* 67:445 1964
26. EPSTEIN, F.H. OSTROM, L.D. JR. JOHNSON, R.C. PAYNE, M.W. HAYNES, N.S. KELLER, J.P. & FRANCES, T.H. Epidemiological studies of cardiovascular disease in total community Tecumseh, Michigan. *Am. J. Med. Sci.* 161:170, 1968
27. EPSTEIN, F.H. Hyperglycemia, A risk factor in coronary heart disease. *Circulation* 38:608, 1967
28. EPSTEIN, F.H. Risk factors in coronary heart disease environmental and hereditary influences. *Br. J. Med.* 30:394, 1969
29. FARAR, Z. "Risk factor" in ischaemic heart disease. *Acta Cardiol. suppl.* 15:7 1972.
30. FITCHER, C.M. & HORN, D. Smoking and health. *WHO Chronicle*, 34:344, 1970.
31. FORDMAN, O. & LINDBLAD, R. The post coronary patient multidisciplinary investigation of middle-aged Swedish males. *J. Psychosom. Res.* 3:82, 1968.
32. FOWLER, R.O. Clinical diagnosis. *Circulation* 46:1079 1972.
33. FRANK, C.W. WEISLATT, E. SHAPIRO, S. & SAGER, R.O. Myocardial infarction in man. Role of physical activity and smoking in incidence and mortality *JAMA* 198:1341, 1968.
34. FREDRICKSON, D.S. & LEE, R.S. A system for phenotyping hyperlipoproteinaemia. *Circulation* 31:331, 1968.
35. FREDRICKSON, D.S. Metast. hyperlipoproteinaemia, and coronary artery disease. *Br. Med. J* 2:187 1971.
36. FRIED, L. CEDERLÖF, R. LINDBLAD, T. & OLSSON, H. Mortality in smoking discordant monozygotic and dizygotic twins. A study on the Swedish Twin Registry. *Arch. Environ. Health* 21:403, 1970
37. FRIED, L. CEDERLÖF, R. LINDBLAD, T. & OLSSON, H. Mortality in twins in relation to smoking habits and alcohol problems. *Arch. Environ. Health* 27:284 1972.
38. FRIEDMAN, M. & ROSENMAN, R.H. Association of specific overt behavior pattern with blood and cardiovascular findings. *JAMA* 169:1274, 1969
39. FRIEDMAN, M. & ROSENMAN, R.H. Symposium on atherosclerotic heart disease The present management of the coronary-prone individual. *Geriatrics* 27:74 1972
40. FROMENT, R. GUINET, P. VIGNON, G. & ROEL, M. Anger coronariac atherosclerosis, à debut précoce et à évolution parallèle chez deux jumeaux. *Arch. Mal. Coeur* 38:263, 1968.
41. GEUDA, L. & FOGEL, D. On the genetic regulation of blood cholesterol. (A study of 60 MZ twin pairs and 50 DZ pairs). *Acta Genet. Med. Gemellol. (Roma)* 9:158, 1960.
42. GEUDA, L. Twins in history and science. Charles C. Thomas, Springfield, Ills., 1961
43. GEUDA, L. & FRENCH, G. Heredity of biological time and population genetics. Diabetes, type 1 diabetes, and peptic ulcer. *Acta Genet. Med. Gemellol.* 19:403, 1970
44. GERTLER, M.M. & WHITE, F.H. Coronary heart disease in young adults multidisciplinary study. Cambridge, Mass. 1964 Harvard University Press.
45. GILL, A. The method of co-twin control. *Science* 96:445, 1963
46. GIBBS, F.L. HOLT, D.E. WHITEMAN, M.W. & SINGH, M.D. Myocardial infarction in twenty-year-old identical twins. *Am. J. Cardiol.* 16:132, 1965
47. GOODMAN, J.W. DELALLA, O. GLAZIER, F. FREEMAN, W.K. LINDGREN, F.T. MICHOLS, A.V. STRIPOWER, R. & TAMPLIN, A.R. Serum lipoprotein transport system in health, metabolic disorders, atherosclerosis and coronary heart disease. *Plasma* 2:118 1964
48. GORDON, T. & KAWNE, W.R. Premature mortality from coronary heart disease. The Framingham Study. *JAMA* 218:1617 1971
49. GRANT, W.C. WASSERMAN, F. MODERNEY, P.L. & THOMPSON, R.V. The incidence of myocardial infarction in portal cirrhosis. *Ann. Intern. Med.* 61 774 1969

- 90 GRÖNQVIST A, LARSSON T & JUNG J Diabetes in Sweden. A clinico-statistical, epidemiological and genetic study of hospital patients and death certificates. *Acta Med. Scand.*, suppl. 477 1967
- 91 GUSTAFSSON, A. ELMFELDT D WILHELMSEN, L. & TIBBLIN, G Serum lipids and lipoproteins in men after myocardial infarction compared with representative population sample. *Circulation* 46:709, 1972.
- 92 deHAAS, J H. Changing mortality patterns and cardiovascular diseases. *Excerpta Medica*, 1966
- 93 HAGBERG L. & FROM HANSEN, P : 50-års undersøgelsen i Glostrup 1966 68 *Ugeskr. Læger* 130:1148, 1968.
- 94 HAMMOND E.C. Smoking in relation to the death rates of one million men and women. *Nat. Cancer Inst. Monogr.* 19:137 1966.
- 95 HAMMOND E.C. GARFINKEL, L. & SEIDMAN, H. Longevity of parents and grandparents in relation to coronary heart disease and associated variables. *Circulation* 43:31 1971.
- 96 HARVALD B. & HAUGE, M. Heredity in goiter and hyperthyroidism. *Acta Med. Scand.* 163:947 1965.
- 97 HARVALD B. & HAUGE, M. Hereditary factors elucidated by twin studies. *Genetics and the Epidemiology of Chronic Disease* U.S. Department of Health, Education and Welfare, Washington, 1968
- 98 HAUGE, M. HARVALD, B. FISCHER, M. GOTLIEB-JENSEN, N. JUEL NIELSEN, N. RANBY, L. SHAPIRO R. & VIDEECK, T The Danish twin register *Acta Genet. Med. (Roma)* 17:315, 1962.
- 99 HAWK, E. R. OSER, R. L. & SUMMERSON, W H. *Practical Physiological Chem.* 12:584 1964
- 100 HILANDER E. Om bekvæmme og fyldeskrift, mindstil hos tvillingar. *Socialmed. tidsskr.* 49:655, 1972.
- 101 HILFANT R.M. BAKKA, V S. deVILLA, M.A. FINE, R. KANDE, V & MEISTER, R.G Use of bicycle ergometry and sustained handgrip exercise in the diagnosis of presence and extent of coronary heart disease. *Br Heart J* 36:1321, 1973.
- 102 HEYDEN, E. BARTZ, A.G. TANEH, E. CASSEL, J.C. TYMOLER, H.A. CORONOW, J.C. & HANSEN, C.G : Angina pectoris and the Rose questionnaire. *Arch. Intern. Med.* 129:961 1971
- 103 HOKLE, L.E. Jr WHITNEY L.H. LEHMANN E.W. DUNN, J. BENJAMIN, B. KING R. PLAKIN, A. & FLEHINGER, R. Occupation, education, and coronary heart disease. Risk is influenced more by education and background than by occupational experiences. In the Bell System. *Science* 161:233, 1968
- 104 HOFFERDAHL, E. & HELMETS, C. Ischemisk hjertesygdom i olika socio-ekonomiska grupper. *Socialmed. tidsskr.* 50:196, 1973.
- 105 HOLMSTEN, A. & STRANDELL, T On the use of chest-head leads for recording of electrocardiogram during exercise. *Acta Med. Scand.* 180:87 1961.
- 106 HUMERFELT S.B. An epidemiological study of high blood pressure with special reference to the influence of blood pressure and age on cardiac signs and symptoms. *Acta Med. Scand.*, suppl. 407 1962.
- 107 International statistical classification of diseases, injuries and causes of death 1968 revision adapted for indexing of hospital records and mortality statistics. Stockholm, Civilttryck AB, 1969
- 108 JENKINS, C.D. MORSEMAN, R.H. & ZYZANSKI, S.J Cigarette smoking. Its relationship to coronary heart disease and related risk factors in the Western Collaborative Group Study *Circulation* 39:1140, 1969.
- 109 JENKINS, C.D. Psychologic and social precursors of coronary disease: Part of two parts. *N. Engl. J. Med.* 284:444 1971
- 110 JENKINS, C.D. Psychologic and social precursors of coronary disease. Second of two parts. *N. Engl. J. Med.* 284:377 1971.
- 111 JENSEN I. BLACKBURN, D R. CHEN, R.P. STURGEON, P & WARE, A.G Serum lipids and serum uric acid in human twins. *J. Lipid Res.* 6:193, 1965.
- 112 JONSSON, A. A method for the determination of the heart size by telecardiography (a heart volume index). *Acta Radiol.* 20:325, 1939.
- 113 JOSLIN, E.P. SCOT R. WHITE, P & MARKS, A. Treatment of diabetes mellitus. Lea & Febiger. Philadelphia, 1969
- 114 KÄHLER O.H. & WENNER, R. Zur Erbpäthologie von Herz- und Kreislaufkrankungen. Untersuchungen an einer autosomalen Zwillingsfamilie. II. Coronarsclerose. *Z. Klin. Med.* 137:507 1940
- 115 KAU I. Alcoholism in twins. Studies on the etiology and sequelae of abuse of alcohol. Almqvist & Wiksell. Stockholm, 1961.
- 116 KANNEL, W B. DAWEY, T R. KAGAN, A. REVOTIKIS, N. & STOKES, J Factors of risk in the development of coronary heart disease six-year follow-up experience. *Ann. Intern. Med.* 55:23, 1961.
- 117 KANNEL, W B. The Framingham Heart Study: Habits and coronary heart disease. U.S. Public Health Service Publ. No. 1515, 1966.

111. KANDEL, W B, LeBAUER, E J, DAWBER, T R, & McNAMARA, P M. Relation of body weight to development of coronary heart disease. The Framingham study. *Circulation* 36:734, 1967.
112. KANDEL, W B, CASTELLI, W P & McNAMARA, P M. The coronary profile: 12-year follow-up in the Framingham study. *J. Occup. Med.* 9:811, 1967.
113. KANDEL, W B, GORDON, T, CASTELLI, W P & MARGOLIS, J R. Electrocardiographic left ventricular hypertrophy and risk of coronary heart disease. The Framingham study. *Ann. Intern. Med.* 71:813, 1970.
114. KANDEL, W B, CASTELLI, W P, GORDON, T & McNAMARA, P M. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. *Ann. Intern. Med.* 74:1, 1971.
115. KANDEL, W B, GORDON, T & SCHWARTZ, M. J. Systolic versus diastolic blood pressure and risk of coronary heart disease. The Framingham study. *Am. J. Cardiol.* 27:336, 1971.
116. KANDEL, W B, & FEINLEIB, M. Natural history of angina pectoris in the Framingham study. Prognosis and survival. *Am. J. Cardiol.* 29:184, 1972.
117. KAWCZYK, M. J. Physical inactivity. In *Preventive Cardiology* p. 163, 1972.
118. KAWCZYK, A, KALLIO V & FORSTROM, J. The significance of psychic and socio-economic stress and other modes of life in the etiology of myocardial infarction. *Ann. Med. Intern. Fenn. suppl.* 143, 1963.
119. KEMP, H G, ELIOT W C, & GORLIN, R. The anginal syndrome with normal coronary arteriography. *Trans. Am. Ass. Physicians* 80:59, 1967.
120. KETZ, A, & BLACKBURN, H. Background of the patient with coronary heart disease. *Prog. Cardio. Dis.* 6:14, 1963.
121. KETZ, A, Ed. Coronary heart disease in seven countries. *Circulation suppl.* 1. of 41, 1972.
122. KETZ, A, ARAVANIS, C, BLACKBURN, H, van BUCHEN, F S P, BOZINA, R, DORDEVIC, B. S, FIDANZA, F, KAWCZYK, M. J, MENOTTI, A, PUDDU V & TAYLOR, H. L. Coronary Heart Disease. Overweight and obesity as risk factors. *Ann. Intern. Med.* 77:18, 1972.
123. KULLER, L, LINDFELD, A, & FISHER, R. Epidemiological study of sudden and unexpected deaths due to atherosclerotic heart disease. *Circulation* 34:1060, 1964.
124. KULLER, L. Sudden and unexpected non-traumatic deaths in adults: review of epidemiological and clinical studies. *J. Chronic Dis.* 19:1162, 1966.
125. Leading article. Congenital hyperlipoproteinaemias and ischaemic heart disease. *Lancet* 1:117, 1971.
126. LEE, R. E, CABELLO, G P, ROSENBERG, L. H, & BACH, F T. Myocardial infarction in one of pair of twenty-seven year old identical male twins. *Am. J. Med.* 34:741, 1963.
127. LEVICHKIN, Z, & SURAWICZ, B. Characterization of true-positive and false-positive results of electrocardiographic Master two-way exercise tests. *N. Engl. J. Med.* 284:811, 1963.
128. LEVITZ, L. B, & ZAK, R. Automated determination of serum total cholesterol. *Clin. Chim. Acta*, 10:381, 1964.
129. LINDOFF W, SEGAL, R. L, & KASPARIAN, H. Paradox of normal selective coronary arteriograms in persons considered to have unimpaired coronary heart disease. *N. Engl. J. Med.* 278:1082, 1967.
130. LINQTON, L. Coronary heart disease in male twins. Hereditary and environmental factors in concordant and discordant pairs. *Acta Med. Scand. suppl.* 511, 1970.
131. LINDMAN, T. Smoking in relation to coronary heart disease and lung function in twins. A co-twin control study. *Acta Med. Scand. suppl.* 435, 1968.
132. LINDMAN, T, LINQTON, L, CEDERLOF, R, & FINEBO, L. The validity of the questionnaire diagnosis. *Arch. Environ. Health* 23:197, 1971.
133. MATTINGLY T W. The postexercise electrocardiogram. Its value in the diagnosis and prognosis of coronary arterial disease. *Am. J. Cardiol.* 9:336, 1968.
134. McDONOUGH, J R, HAMER, C. G, GREENBERG, B. G, GRIFFIN, L. H. Jr, & EDWARDS, A. J. Jr. Observations on serum cholesterol levels in the twin population of Evans County Georgia. *Circulation* 23:908, 1961.
135. McKUSICK, V. A, & MURPHY, E. A. Genetic factors in the etiology of myocardial infarction. In James, T. M, & Keys, L. W. editors. The etiology of myocardial infarction. Boston, 1963. Little, Brown and Company.
136. McKUSICK, V. A. Physiological statistics. Sec. Ed. Wiley New York, 1965.
137. Medical Research Council Committee on the Aetiology of Chronic Bronchitis. Standardized questionnaires on respiratory symptoms. *Br. Med. J.* 2:186, 1960.
138. MITTER, K. Serum cholesterol and heredity. A twin study. *Acta Med. Scand.* 171:481, 1962.
139. MULL, W E. Heredity and hypertension. *Practitioner* 207:10, 1971.

- 147 MOSER, A. More subtle. *Likartidningen* 66:1011, 1969
- 148 MORIYAMA, I. M., KRUEGER, D. E. & STAMLER, J. *Cardiovascular diseases in the United States*. Cambridge, Mass. Harvard University Press, 1971
- 149 MULLICANT III, HICKEY, M. & MAURER, R. Coronary heart disease in women. Study of risk factors in 100 patients less than 60 years of age. *Circulation* 36:877 1967
- 150 MYERS, A. R., EPSTEIN, F. H., DODGE, R. J. & MIKKELSEN, W. M.: The relationship of serum uric acid to risk factors in coronary heart disease. *Am. J. Med.* 45:820 1968.
- 151 MYRHED, M.: Alcohol consumption in relation to factors associated with ischemic heart disease. A co-twin control study. *Acta Med. Scand. suppl.* 567 1974
- 152 MAPER, J. A., MITZNER, H. & JOHNSON, R. C. Limitations of morbidity and mortality data obtained from family histories. Report from the Tecumseh Community Health Study. *Am. J. Public Health* 63:30 1971.
- 153 NEWLAND, H. Antagonism of the anti thrombotic effect of warfarin by uric acid. *Am. J. Med. Sci.* 176:44, 1968.
- 154 MIKKILÄ, E. A. & AHO, A. Family study of serum lipids and lipoproteins in coronary heart-disease. *Lancet* 1 964 1973.
- 155 NOBLE, R. P. & CAMPBELL, F. M. Improved accuracy in automated fluorometric determination of plasma triglycerides. *Chn. Chem.* 16:166, 1970
- 156 OLIVER, M. F. & STUART-HARRIS, C. H. Present position concerning prevention of heart disease. *Br. Med. J.* 2:1303, 1965.
- 157 OSBORNE, R. H., ADLERSBERG, D., DeGORGES, F. V. & WANG, C. Serum lipids, heredity and environment. A study of adult twins. *Am. J. Med.* 36:64, 1969
- 158 OSBORNE, R. H. & DeGORGES, F. V. Genetic basis of morphological variation. A evaluation and application of the twin study method. Harvard University Press, Cambridge, Mass. 1969
- 159 OSBORNE, R. H., DeGORGES, F. V. & MATHERS, J. A. L. The stability of blood pressure. Basal and casual measurements in adult twins. *Am. Heart J.* 66:176, 1963
- 160 OSTRANDER, L. D. Jr., FRANCIS, T. Jr., HAYNER, M. E., KJELSTROM, M. O. & EPSTEIN, F. H. The relationship of cardiovascular disease to hyperglycemia. *Ann. Intern. Med.* 62:1168, 1965.
- 161 PARADE, G. W. & LEHMANN, W.: Angina pectoris bei Erbgelichen Zwillingen. *Klin. Wochn.* 17:1036, 1938.
- 162 PAUL, O. & SCHATZ, M. On sudden death (Editorial). *Circulation* 43:7 1971
- 163 PEDGORE, L. S. The general background of common diseases. *Acta Genet.* 4:287 1963.
- 164 PUNAR, S. & KAJVONEN, M. J. Angina pectoris and ECG abnormalities in relation to prognosis of coronary heart disease in population studies in Finland. In early diagnosis of coronary heart disease. *Adv. Cardiol.* 8: 143, 1973. (Karger, Basel).
- 165 RAHE, R. H. Life crisis and health change. From Mary Philip R. A. and Wittenborn, J. R. Psychotropic drug responses. Advances in prediction, 1969. Courtney O. Charles C. Thomas, Springfield, Ill.
- 166 RAHE, R. H. & LIND, E. Psychosocial factors and sudden cardiac death: A pilot study. I. *Psychosom. Res.* 15:119 1971
- 167 READER, R. & WYNN, A. The increasing mortality from coronary heart disease in men, 1960 to 1966. *Med. J. Aust.* 2:740 1966.
- 168 REDWOOD, D. R. & EPSTEIN, S. R. Uses and limitations of stress testing in the evaluation of ischemic heart disease. *Circulation* 46:1113 1972
- 169 RIFKIND, R. M., BOYLE, J. A., CALE, M., GREIG, W. & BUCHANAN, W. W. Study of serum lipid levels in twins. *Cardiovasc. Res.* 2:148, 1968.
- 170 ROSE, G. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. *Bull. WHO* 27 648 1962
- 171 ROSE, G. Familial patterns in ischaemic heart disease. *Br. J. Prev. Soc. Med.* 16:76, 1964
- 172 ROSE, G. & SLACKMURN, H. Cardiovascular survey methods. WHO Monograph, series, No. 86, 1965.
- 173 ROSE, G. Predicting coronary heart disease from minor symptoms and electrocardiographic findings. *Br. J. Prev. Soc. Med.* 25:94, 1971
- 174 ROSE, G. Smoking and cardiovascular disease. *Am. Heart J.* 85:838, 1973.
- 175 ROSENTHAL, R. H., FRIDMAN, M., STRAUS, R., JENKINS, C. D., ZYZANSKI, S. J. & WURM, M.: Coronary heart disease in the Western Collaborative Group Study. A follow-up experience of 4 1/2 years. *J. Chronic Dis.* 23:173, 1970

174. Royal College of Physicians. Smoking and health now. London: Pitman Medical and Scientific Publishing Co, Ltd, 1971.
175. KATZ, R.L. & ZOHMAN, H.L.: Relative significance of heredity, diet, and occupational stress in coronary heart disease of young adults. *Am. J. Med. Sci.* 236:268, 1958.
176. SALES, E.M. & HOUSE, J. Job dissatisfaction as possible risk factor in coronary heart disease. *J. Chron. Dis.* 24:82, 1971.
177. SMITZ, C.C. An evaluation of the effect of smoking on coronary heart disease. I. Epidemiological evidence. *JAMA* 223:192, 1968.
178. SHAWY, H.M., LITTLE, A., MURPHY, E.A. & RYKERT, H.E. Studies of male survivors of myocardial infarction due to "essential" atherosclerosis. I. Characteristics of the patients. *Can. Med. Assoc. J.* 84:19, 1961.
179. SUD, L.L., SARAHANA, A.A. & LITTMANN, D. Coronary-artery disease in identical twins. A family study. *Br. J. Med.* 274:55, 1966.
180. SVEN, I., BLOMQUIST, G. & MÖRCK, G. Studies on myocardial infarction in Malmö 1935 to 1964. V. Some clinical data with particular reference to diabetes, myocardial and heart rupture. *Acta Med. Scand.* 169:16, 1961.
181. SVEN, I. Myocardial infarction. Clinical features and outcome in three thousand thirty-six cases. *Acta Med. Scand. suppl.* 440, 1962.
182. EDWARDS, D.W. The status of risk factors and coronary heart disease. *J. Chron. Dis.* 23:515, 1970.
183. BLACK, L. & EVANS, K.A. The increased risk of death from ischaemic heart disease in first degree relatives of 121 men and 96 women with ischaemic heart disease. *J. Med. Genet.* 9:229, 1972.
184. STAMLER, J. & EPSTEIN, F.H. Coronary heart disease: Risk factors as guides to preventive action. *Prev. Med.* 1:27, 1972.
185. STAMLER, J. Epidemiology of coronary heart disease. *Med. Clin. North Am.* 67:1, 1973.
186. STAM, F.J. Myocardial infarction in patients with portal cirrhosis. *Med. Rev.* 19:37, 1961.
187. STRANDHILL, T. Circulatory studies on healthy old men, with special reference to the limitation of the maximal physical working capacity. *Acta Med. Scand. suppl.* 414, 1966.
188. SLEGER, O. & KOLLER, F. Angina pectoris, myocardial infarction and hypercholesterolemia in erythrocytic twins. *Helv. med. Acta* 24:320, 1961.
189. SUNDT, F. Alcoholism and mortality. *Universitetsforlaget*, Oslo, 1967.
190. SYME, S.L., HYMAN, M.M. & ENTERLINE, P.E. Some social and cultural factors associated with the occurrence of coronary heart disease. *J. Chronic Dis.* 17:577, 1964.
191. TACKENBOM, J. Anthropometric, electrocardiographic and blood pressure studies on adult male twins. *Ann. Acad. Sci. Fenn. (Med)* 107, 1964.
192. The Health Consequences of Smoking. A report to the Surgeon General, 1972. US Dept of Health, Education and Welfare, 1972.
193. The use of twins in epidemiological studies. Report of WHO meeting of Investigators. *Acta Genet. Med. Gemellol. (Roma)* 16:109, 1968.
194. THOMSEN, H. Die Erbiologie des Diabetes Mellitus. Vorläufiges Ergebnis der Zwillingsuntersuchungen. *Arch. f. Kaiser-u. Ges. Med.* 33:189, 1938.
195. THORELL, T. Psychosocial factors in relation to the onset of myocardial infarction and to some metabolic variables. Pilot study. Department of Medicine, Södersjukhuset Hospital, Karolinska Institute, Stockholm, 1971.
196. THOMAS, C.B. & COHEN, S.H. Familial occurrence of hypertension and coronary artery disease, with observations concerning obesity and diabetes. *Ann. Intern. Med.* 43:80, 1955.
197. TIMLIN, G. A population study of 80-year-old men. An analysis of the non-participation group. *Acta Med. Scand.* 175:453, 1968.
198. TIMLIN, G. High blood pressure in men aged 55. A population study of men born in 1913. *Acta Med. Scand. suppl.* 470, 1967.
199. TIMLIN, G. & WILHELMSEN, L. Amerokärls sjukdomsfaktorer (I) Hjärtinfarktens riskfaktorer. *Läkartidningen* 66:1738, 1971.
200. TIMLIN, G. Primär och sekundära riskfaktorer. *Läkartidningen* 66:648, 1972.
201. TIMLIN, G. Risk factors for developing myocardial infarction and other diseases. The men born in 1913 study in preventive cardiology (eds. G. Tibblin, A. Keys & L. Wedel), pp 33-42. Almqvist & Wiksell Förlags AB, Stockholm, 1972.

204. TRUETT J, CORNFIELD J & KAMEL, W : A multivariate analysis of the risk of coronary heart disease in Framingham, *J Chronic Dis*, 20:511 1967
205. Twin registries in the study of chronic disease. With particular reference to the relation of smoking to cardiovascular and pulmonary diseases. Report of an International Symposium in San Juan, Puerto Rico, 1-4 Dec. 1969 H. Cederlöf, P H. Extein, L. Friberg, Z. Hrubec, E.P. Radford, *Acta Med. Scand*, suppl. 823, 1971.
206. VEDIN, A.I. WILHELMSSON, C., BOLANDER, A. M & WERKÖ, L. Mortality trends in Sweden 1961-1968 with special reference to cardiovascular causes of death, *Acta Med. Scand*, suppl. 818, 1971.
207. VEDIN, A.I. WILHELMSSON, C., KIMFELDT H, TIBBLIN, G, WILHELMSSON L, & WERKÖ, L.; Sudden death: Identification of high risk groups, *Am. Heart J* 80:124, 1973.
208. VERSCHUER, O. von.; Die Zwillingsforschung in Dienste der Inneren Medizin, *Verh. Dtsch. Ges. Inn. Med.*, 94:202, 1968
209. WAHLBERG F : Intravenous glucose tolerance in myocardial infarction, angina pectoris and intermittent claudication, *Acta Med. Scand*, suppl. 463, 1966.
210. WARDWELL, W L, HYMAN, M, & RAHNSON, C R.; Socio-environmental antecedents to coronary heart disease in 67 white males, *Sci. Sci. Med.*, 8:103, 1962.
211. WESTLUND, K, & NICOLAYSEN R.; Ten-year mortality and morbidity related to serum cholesterol. A follow-up of 3,781 men aged 40-49 *Scand. J Clin. Lab. Invest.*, suppl. 187 1972
212. WHITE, P D : The genes, the heart and destiny *N. Engl. J Med*, 236:905, 1967
213. WHITE, P D : The importance of heredity in coronary heart disease, *Circulation* 32:296, 1966
214. WIKLAND B.; Medically unattended fatal cases of ischaemic heart disease in a defined population, incidence during one year in Stockholm with particular reference to prevalence of certain previously diagnosed disorders, some characteristics of the last attack, and post-mortem findings, *Acta Med. Scand*, suppl. 834 1971
215. WILHELMSSON, L, WEDEL, H, & TIBBLIN, G : Multi-cause analysis of risk factors for coronary heart disease, *Circulation* 48:860, 1973.
216. ÅSTRAND, L. Exercise electrocardiograms recorded twice with an 8-year interval in a group of 804 women and men 48-65 years old, *Acta Med. Scand*, 178:377 1968.
217. ÅSTRAND L, and co-workers in the Scandinavian Committee on ECG classification. The "Minnesota code" for ECG classification. Adaptation to CR leads and modification of the code for ECGs recorded during and after exercise. By the Scandinavian Committee on ECG classification, *Acta Med. Scand*, suppl. 461 1967
218. ÅSTRAND, L, & LUNDMAN T : The exercise electrocardiogram in coronary heart disease its prognostic value, *Scand. J Clin. Lab. Invest.*, 22:301, 1968.
219. ÅSTRAND L. Exercise ECG its prognostic significance, *Adv. Cardiol.*, 8:143, 1973.

APPENDIX

The "Minnesota code" for ECG classification Adaptation to CR (CH) lead and modification of the code for ECG recorded during and after exercise

Q and QS patterns

(Do not code in presence of ventricular conduction defects 6,4 or 7.1)

- 1.1.1 Q/R amplitude ratio 1/3 or more plus Q duration 0.03 sec. or more in any of leads I, II, CR₁ 3,4,5,6,7
- 1.1.2 Q duration 0.04 sec. or more in any of leads I, II, CR₁ 3,4,5,6,7
- 1.1.3 Q duration 0.04 sec. or more plus R amplitude of 3 mm or more in lead VL
- 1.1.4 Q duration 0.03 sec. or more in lead III plus any Q wave of at least 1.0 mm amplitude in VT
- 1.1.5 Q duration 0.04 sec. or more in lead VF
- 1.1.6 QS pattern when R wave is present in adjacent lead to the right on the chest in any of leads CR₂ 3,4,5,6,7
- 1.1.7 QS pattern in all of leads CR₁ through CR₄ 5,6 or 7
- 1.2.1 Q/R amplitude ratio 1/3 or more plus Q duration at least 0.02 sec. and less than 0.03 sec. in any of leads I, II, CR₂ 3,4,5,6,7
- 1.2.2 Q duration at least 0.03 sec. and less than 0.04 sec. in any of leads I, II, CR₂ 3,4,5,6,7
- 1.2.3 QS pattern in lead II
- 1.2.4 Q duration of at least 0.04 sec. and less than 0.06 sec. in lead III plus any Q wave of at least 1.0 mm amplitude in VT
- 1.2.5 Q duration at least 0.04 sec. and less than 0.06 sec. in lead VF
- 1.2.6 Q amplitude of 3 mm or more in either of leads III, VF
- 1.2.7 QS pattern in all of leads CR₁ through CR₃
- 1.2.8 R amplitude decreasing to 2.5 mm or less, and absence of codes 3.2, 7.2 or 7.3 between any of leads CR₁ and CR₂, CR₂ and CR₃, CR₃ and CR₄, CR₄ and CR₅, CR₅ and CR₆ or CR₆ and CR₇
- 1.3.1 Q/R amplitude ratio at least 1/3 and less than 1/3 plus Q duration of at least 0.02 sec. and less than 0.03 sec. in any of leads I, II, CR₂ 3,4,5,6,7
- 1.3.2 QS pattern in absence of code 3.1, in each of leads CR₁ and CR₂
- 1.3.3 Q duration of at least 0.03 sec. and less than 0.04 sec. plus R amplitude of 3 mm or more in lead VL
- 1.3.4 Q duration of at least 0.03 sec. and less than 0.04 sec. in lead III plus any Q wave of at least 1.0 mm amplitude in lead VF
- 1.3.5 Q duration of at least 0.03 sec. and less than 0.04 sec. in lead VF
- 1.3.6 QS pattern in each of leads III and VF

ST depression

(Do not code in presence of ventricular conduction defects 6,4 or 7.1,2,4)

- | | | |
|-----|--|-------------------------------------|
| 4.1 | ST J depression of 1.5 mm or more
or 1.0 mm or more
and ST segment straight and slowly ascending, horizontal or downward sloping | CR ₂ 7
I, II, VL, VF |
| 4.2 | ST J depression of 1.0-1.4 mm and ST segment straight and slowly ascending,
horizontal or downward sloping | CR ₂ 7 |
| 4.3 | ST J depression of 0.5-0.9 mm and ST segment straight and slowly ascending
horizontal or downward sloping | CR ₂ 7
I, II, VL, VF |
| 4.4 | No ST J depression as much as 0.5 mm but ST segment downward sloping and
reaching 0.5 mm or more below P-R baseline | CR ₂ 7
I, II, VL, VF |
| 4.5 | No ST J depression as much as 0.5 mm but ST segment horizontal or downward
sloping but reaching less than 0.5 mm below P-R baseline | CR ₂ -7
I, II, VL, VF |
| 4.6 | Isolated ST J depression of 1.5 mm or more
or 1.0 mm or more,
ST segment upward sloping | CR ₂ 7
I, II, VL, VF |
| 4.7 | Isolated ST J depression of 0.5-1.4 mm or more
or 0.5-0.9 mm or more,
ST segment upward sloping | CR ₂ 7
I, II, VL, VF |

Supplementum 579

Cardiac Catheterization

*Development of the Technique Its Contributions
to Experimental Medicine, and its Initial Applications in Man*

André Cournand

Acta Medica Scandinavica

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CARDIAC CATHETERIZATION

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Medicine and its Initial Applications in Man

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CARDIAC CATHETERIZATION

In considering the successive phases of development of the technique of cardiac catheterization, which in modern times has played an important role in experimental and human physiology as well as in medical practice three separate periods may be identified.

I The first extends from the middle to the end of the 19th century. After a probe—a tube of glass or flexible material—introduced into animal hearts was used to solve two controversial problems in cardiac physiology the technique was extended to improvement of recording measurements and knowledge of cardiac events and mechanisms. This experimental period yielded a considerable amount of information concerning the technique itself, the required instrumentation and the validity of the physiological information derived from it. Briefly, catheterization of the heart provided a strong impulse to the development of cardiac physiology in various animal species.

II The second period started after a lag of 35 years in 1929 with the courageous and repeated attempts by a young surgeon still in training to demonstrate on himself that a ureteral catheter could be passed through an arm vein downstream to the right atrium. After these early tests of the apparent safety of right atrial catheterization in man several investigators utilized the technique as a means of injecting undiluted a radiopaque substance into the right atrium in order to visualize the pulmonary circulation and heart chambers. In addition a few other clinicians among them Jimenez Diaz in isolated attempts and without follow-up employed the catheter to draw samples of mixed venous blood from the right atrium for analysis of some of its chemical contents in particular respiratory gases. Apart from the interest this use of the catheter aroused in the early development of angiocardiology as a clinical method of diagnosis it had negligible influence on, and signifi-

cance for progress in knowledge of fundamental or applied cardiac physiology during the remaining few years of this period.

III The third period, starting in 1936, witnessed the elaboration, extension, and universal acceptance of intravascular and intracardiac catheterization as an essential method of physiological investigation in normal and diseased man of diagnosis in cardiocirculatory (acute chronic, acquired or congenital) and pulmonary diseases and of controlled testing and evaluation of a variety of medical and surgical treatments of these diseases. This transformation resulted from several developments. (a) Design and fabrication of the catheter were improved. (b) It was found that a vast array of measuring equipment and tracer substances could be productively used in conjunction with catheterization. (c) It was repeatedly demonstrated that the catheter could be left *in situ* for long periods of time. (d) The ability to advance its tip into the right ventricle and pulmonary artery opened up the study of the pulmonary circulation in various physiologic conditions and in cardiopulmonary diseases. (e) The feasibility of advancing the catheter tip into other cardiac chambers and large vessels either through abnormal communications or through channels other than large peripheral veins was demonstrated. During the past 33 years access to human intracardiac cavities and intravascular lumina by means of the catheter led to far reaching developments in physiologic knowledge and medical practice based upon this knowledge.

In this essay I shall analyze in some detail the history of the first two periods. The third period is the subject of an independent examination which I am now in the process of completing since it is deserving of as searching a treatment as the previous periods. Accordingly I shall consider here in some detail the investigations and the plan on which they were based which marked the preceding and early years of this period (1932–1945) but will restrict myself to a few general comments regarding the proliferating applications of cardiac catheterization which characterize the subsequent portions of this period.

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I THE ADVENT OF CARDIAC

CATHETERIZATION—CLAUDE BERNARD

Cardiac catheterization was first performed (and also named) so far as can be ascertained by Claude Bernard the nineteenth century scientist who so particularly stressed the importance of physiology for the understanding of disease. A good medical school cannot be separated from the hospital. "I he wrote reflecting his conviction that the backbone of scientific medicine is experimental physiology integrated with clinical observation.

The problem which evoked the novel procedure of introducing a measuring device into the beating heart in order to make scientific observations concerned the respective temperatures of blood in the right and left ventricles. In the 1840's this was a matter of considerable controversy. Several decades earlier Lavoisier had suggested that animal heat is produced as a result of respiratory gas exchange during transit of blood through the lungs.² Asserting that respiration is a form of combustion he declared that in the lungs the combination of oxygen with carbon produced carbon dioxide and its conjunction with hydrogen water both reactions being accompanied by the liberation of the element caloric that is heat. Subsequently his followers drew the conclusion that the temperature of

blood returning to the left heart is greater than that of blood entering the pulmonary circuit.

For some 50 years invocation of Lavoisier's authority was sufficient to support this view. In 1837 an alternative was advanced by Gustav Magnus.³ Combustion takes place in the tissues. He based this claim on the results of experiments in which he showed that in the venous system the carbon dioxide concentration increased whereas the oxygen content declined as compared with the arterial system. Later in 1842 according to Bernard Magnus stated that carbonic acid is not formed in the lungs and that combustion therefore occurs in all tissues of the organism.

With the issue thus joined the competing hypotheses were vigorously debated. From the pulmonary combustion hypothesis it followed that blood entering the left ventricle is warmer than that flowing into the right-sided chamber. The tissue combustion hypothesis entailed the assertion that blood coming from the tissues by way of the venae cavae is warmer. The first view was upheld by Gay Lussac and Magendie—a professor at the Collège de France—who were planning experimental work on the relative concentrations of carbon dioxide in arteries and veins. These investigators denied the validity of Magnus' findings strictly on the basis of their doubt in the reliability of his work. Observations on the temperature difference between the ventricles were made by others but were of dubious validity since the data were obtained from open-chest or dead animals.

To determine which of the opposing views was correct, Bernard saw that a simple experiment might be performed—provided that the experimental conditions approximated the normal physiological state as closely as possible. In these circumstances measurement of the temperature of the blood in each of the ventricular cavities would be sufficient to determine which hypothesis was correct.

Bernard's initial observations on this problem were made in 1844 when he was serving as an assistant to Magendie. The experimental subject was a horse whose exposed carotid artery had been incised. Through the opening Bernard introduced a

Claude Bernard *Leçons sur la Chaleur Animale* Paris, Librairie J.-B. Baillière et Fils, 1876 p. 3

It was among Lavoisier's principal contributions to show that animal heat is produced through a chemical reaction, and not through vital, mechanical, or other processes. His interest of course was in the relationships among combustion, respiration, and liberation of animal heat, and not so much in the physiological exploration of these processes, and he granted the possibility that combustion takes place elsewhere in the organism than in the lungs. For a discussion of these matters, see Hoff H. E. Grüsslein, R. and Sakai E. Claude Bernard on animal heat. An unpublished manuscript and some original notes. *Perspectives in Biology and Medicine* 7: 347-368, 1965.

Magnus, G. Über die im Blute enthaltenen Gase Sauerstoff, Stickstoff und Kohlensäure. *Ann. Phys. Chem.* 1... 583 (1837).

Op. cit. p. 23

Ibid. p. 4

Ibid. p. 4

long mercury thermometer into the left ventricle and recorded the temperature of the blood. Then he passed the thermometer through the jugular vein into the right ventricle. In repeated observations the temperature was a few tenths of a degree higher on the right side, indicating that the pulmonary combustion hypothesis was incorrect. The temperature difference Bernard noted was lesser under basal conditions than during digestion or exercise.

It was characteristic of Bernard's physiologic work that he returned repeatedly to a given problem, sometimes to confirm or to refute the observations of other scientists, at other times to repeat the work using better techniques frequently it was Bernard himself who contributed the improved methods. Over a period of 30 years Bernard returned to the temperature problem not only in 1849 and 1853-1855 but also in 1857 and 1871-1872.

In 1849 before the members of the Société de Biologie Bernard presented an experiment showing

Did p. 42. Bernard recorded the fact that his experimental subject, which had been made available to him by Magendie, then chairman of commission on horse hygiene had glanders. This condition was likely not in an advanced stage, however and therefore probably did not distort the intraventricular temperature differences, since the horse was able to perform usual activities at the time Bernard studied him.

A useful guide to Bernard's scientific work is provided by M.D. Grmek, *Catalogue des Manuscrits de Claude Bernard* (Paris, Masson et Cie, 1967). According to this text, records of some of the numerous studies of the temperature problem appear in Notebooks 7b (1848-1849), 8c (1854-1855), 8f (1853) and 26e (whose 50 folios contain complete transcripts of all experiments on the temperature problem).

Bernard op. cit. p. 42.

¹¹ Liebig, G. *Über die Temperaturunterschiede des venösen und arteriellen Blutes*. Gießen, 1853.

See Recherches sur l'influence que la section du grand sympathique exerce sur la chaleur animale (*Compte rend. de l'Académie des Sciences* 1852; *Leçons de physiologie expérimentale appliquée à la médecine* (Paris, 1855) ed. I p. 196; Recherches expérimentales sur la température animale (*Compte rend. de l'Académie des Sciences* 1856 vol. 43); *Leçons sur les propriétés physiologiques et les altérations pathologiques des liquides de l'organisme* (Paris, 1859 vol. I p. 50 et seq.). For Walferdin's description of his instrument, see *Bulletin de la Société géologique* vol. II, p. 83.

¹² Becquerel et Breschet, *Mémoires sur la chaleur animale. Annales de sciences naturelles Zoologie* 7^e série, vol. III and IV.

¹³ Bernard *Chaleur Animale* p. 78. The translations of this and the following two passages are not literal at every point and are my own.

that the blood in the hepatic vein is warmer than that in the central aorta. In the period 1853-1855 after G. Liebig had reached conclusions similar to his ¹¹ Bernard returned on a number of occasions to intracardiac temperature measurement in dogs and sheep collaborating with Walferdin a specialist in temperature measurement. In these experiments a metastatic thermometer devised by Walferdin was used; this instrument could discriminate temperature differences as slight as 0.01°C. The results of these studies were published in several texts. In all instances it appears Bernard's original observations were confirmed.

Bernard's book *Leçons sur la Chaleur Animale* published in 1876 recapitulates 22 lectures on this subject delivered during the academic year 1871-1872. In the first lectures he reviewed the entire early history of the debate concerning the temperature of the blood in the ventricles. In the fifth lecture Bernard reported experiments in which he used a very sensitive thermoelectric probe a modification of the instrument originally developed by Becquerel ¹² like Walferdin's thermometer it allowed differentiation of temperatures by as little as 0.01°C. But what is chiefly of interest in the fifth lecture is Bernard's admirable description of the catheterization technique as applied in intraventricular temperature measurement in a dog:

An incision is made (in the neck) to expose the jugular vein. It is by this route that right heart catheterization will proceed. For this purpose the tip of the catheter is given slight anterior curvature introduced into the vessel and advanced by continuous yet not excessive pressure. Soon we feel a slight resistance. Previous experiments have shown that this occurs when the tip of the catheter has reached the atrium. The occurrence of this resistance therefore provides an important landmark. One must then rotate the probe medially and toward the left while simultaneously withdrawing it short distance. Then, by pushing again, one enters the right ventricle. The goal has been reached.

The second step is to introduce the second catheter into the left ventricle. After exposing and isolating the internal carotid, we introduce the catheter at the same time pulling the vessel upward in order to eliminate the curve that normally is present at the carotid artery's junction with the aorta.

Introduction of the thermoelectric probe into the left ventricle cannot always be accomplished with facility. Most of the time the sigmoid [i.e., aortic] valves prevent the passage of the catheter's tip which tends to become lodged in the pycnon next formed by leaflet and the vessel wall. One cannot advance into the ventricle without perforating or rupturing the leaflet.

After describing the anatomical and functional characteristics of the aortic valvular leaflets Bernard goes on:

In order to introduce the tip into the left ventricle one must wait for the moment when systole begins, as there is then a small aperture between the free edges of the valve leaflets. This is not easy. However it is easy to feel whether the tip of the catheter has entered the left ventricular cavity or is blocked at its entrance by the valve. In the latter case one sees the probe moving upward with each cardiac contraction. If one holds the proximal end of the catheter between two fingers one feels a single impact and senses that the tip of the catheter has met an obstacle and is not free. In the former case, to the contrary one feels that the contraction is transmitted more forcefully but that there are two distinct impacts, the first coinciding with systole and produced without doubt, by the impact of the contracting ventricular wall on the tip which is struck and pushed aside and the second corresponding to diastole and probably due to the shock of closure of the aortic valve leaflets.

I [our present] experiment fate has favored us, and we have penetrated into the left ventricle. you can see the proximal end of both catheters moving simultaneously in phase with the ventricular contractions. It is necessary to secure the catheters by means of ligatures [proximal to the portals of entry into the vascular system], both to prevent hemorrhage and to avoid ejection of the tips from the cardiac cavities. result of extracardiac contractions but the ligatures are not applied until the tips have been withdrawn slightly in order to avoid the impact of the contracting wall on otherwise the tip would injure the endocardium or eventually perforate the muscle resulting in intrapericardial hemorrhage.¹¹

Bernard went on to consider the position of the experimental subject that best avoids passing the catheter along false routes in the venous system.

In my early experiments on horses I always proceeded with the animal in the standing position.

Later as Buzzs recounts¹² Bernard gave a minutely detailed description of cardiac catheteriza-

¹¹ *Ibid.* pp. 80-81.

¹² *Ibid.* p. 82.

¹³ Buzzs: A. Claude Bernard on cardiac catheterization *Am. J. Cardiol.* 4: 403, 1959.

¹⁴ J. B. Ballière et Fils, Paris, 1879.

¹⁵ See Hoff et al. *op. cit.* (note 1).

¹⁶ *Ibid.*

¹⁷ Experiment 3 in *5 ans d'Essais Contingent Hemorrhagiques* (New York: Hafner Publishing Co. 1964).

¹⁸ Notebook 77 in Grunke, *op. cit.* (note 8).

¹⁹ *Ibid.* Notebook 77 87 and 14th th. later text. written in 1877 contains a number of reports of intracardiac pressure measurement. Document 77 in Folio states that catheterization of the dog heart causes "angular deviation

tion as an operative technique. His text, published posthumously in *Leçons de Physiologie Expérimentale*²⁰ presents the elements of the procedures for both venous and arterial catheterization of the heart, as well as for entry into the great vessels.

The temperature problem had been solved in the 1850's by Bernard and other investigators. Yet Bouillaud a member of Bernard's section of the Académie des Sciences attacked his conclusions in 1877 at a meeting of the Académie. Bouillaud's argument was based on the principle of authority. What Lavoulier had conceived could not be in error. Bernard's response²¹ to Bouillaud is admirable for its detachment and clarity as well as its insistence on controlled observation under conditions approximating the physiological as closely as possible. He was able to define a number of factors that might distort findings in catheterization, and he indicated how these variables might be controlled.

Bernard's utilization of the technique of cardiac catheterization was not confined to the temperature problem. He also recognized the possibility of measuring intracardiac pressures by this means. More than 100 years earlier in 1733 Stephen Hales had had the idea of introducing a glass tube into the venous and arterial systems of a living mare in order to measure pressures.²² However he did not perform catheterization of the heart chambers in living, closed-chest animals.

Bernard's purpose in measuring intracardiac pressures was to study the physiology of the nervous system and its influence upon blood pressure.²³ On November 29, 1847, at the Collège de France he performed his first experiment on this problem. Present as observers were Magendie and Rayer. Through the incised jugular vein of a small dog, Bernard introduced a glass tube into the right ventricle. Upon connecting the external end of the tube to a pressure recording system (cardiodynamometer) which had been developed by Poiseuille he measured the right ventricular pressures. The systolic pressure proved to be much lower than that of the peripheral arteries. At autopsy of the dog, it was seen that the right ventricle had been perforated by the tube, presumably near the end of the experiment, since the final measurements were obviously out of line with the initial and intervening measurements.

Bernard continued his investigations of pressures in the intracardiac cavities intermittently during at least the following seven years. His notebooks²⁴

contain records of experiments on dogs and horses in which intracardiac blood pressures were measured under a variety of physiological states and types of stimulation.

In scanning Bernard's accomplishments related to cardiac catheterization, I note several features:

1 He saw that solution of a scientific problem might be enabled by the development of a totally new laboratory technique

2. He in fact developed the technique and used it successfully in the performance of experiments that resolved the problem and over the course of time he brought the method to a high level of sophistication.

²³ In *De Motu Cordis* chapter II p. 28 (translated by Chauncy Leake, Tercentenary Edition Charles C Thomas, Publisher Springfield Illinois 1928), Harvey refers to "three significant features to be noted in the motion and in the period of movement of the heart" and describes the first of these features. "The heart is lifted and rises up to the apex so that it strikes the chest at that moment [ventricular systole] and the beat may be felt on the outside

Milne Edwards, in a report on two presentations by Chauveau and Marey (*vide infra*) published in *Comptes rendus des Séances de l'Académie des Sciences*, vol LIV April 28, 1862, p 899 refers to Harvey's observations in the following terms.

"The illustrious Harvey studied this phenomenon with his usual insight in order to discover its mechanism; he made a number of experiments in animals, and took advantage of a very rare pathological case which enabled [him] to observe directly the heart of a young man through gaping aperture in the thoracic anterior wall. The facts observed led him to attribute the apical thrust against the sternum and the ribs to the stiffness of the heart muscle wall associated with ventricular systole and its movement of projection depending also on this ventricular systole.

²⁴ The key to the source of Milne Edwards's statement (note 23) was supplied to me by Dickinson W Richards few weeks before his death. In "De Generatione Animalium and the Fetal Assessment" (chapter 10 of the book by Gwenneth Whitteridge *William Harvey and the Circulation*, published by McDonald, London, and American Elsevier Inc., New York in 1971) appears description of Harvey's observations of the beating heart of the eldest son of the Viscount Montgomery

²⁵ In Chapter II of Volume II p 207 of the *Traité d'Anatomie Médicale* (Brouin et Chénede, Paris 1819) R. T. H. Laënnec stated, "The impact of the heart (usually felt only in the precordial region) corresponds with the beginning of the systole of the ventricles. Laënnec clearly distinguished this phenomenon from the somewhat similar appearance rarely produced by atrial contraction.

3 From the beginning he emphasized the importance of making physiological observations under conditions in which the subject was in the basal state and as close to normal as possible. Recognizing inter-species variability he used animals of various species in his efforts to obtain experimental data

4 He brought a variety of experts from other fields into his research when this seemed fruitful significantly enhancing the quality of the observations by doing so

5 Finally he was the first to measure right ventricular pressures in the living animal thus opening a new field to scientific investigation

2. INTRAVENTRICULAR PRESSURE RECORDING

A. The cardiac apex beat—Chauveau and Marey

It would be refreshing to linger with Bernard and his work for the physiologist might be surprised to find attitudes that he imagined to be quite contemporary set forth with great clarity and logical rigor in the work of this outstanding scientist. The requirements of even an overview of the history of cardiac catheterization demand however that we go on to the next important instance of its application. Again the incentive was provided by a physiological controversy—this instance concerning the nature and timing of the cardiac apex beat. This problem and its cognate the question of the variations of the apex beat in disease have played an important role in cardiac semiology.

Two centuries previously Harvey had taken the position that the impulse felt in the apical region of the thorax was due to ventricular contraction and therefore started with ventricular systole.²⁶ Harvey's opinion was later upheld by Laënnec.²⁷ Nevertheless controversy arose as to its exact timing (*vide infra*). Understandably its resolution was of interest not only to physiologists but to physicians as well. In 1861 two French investigators A Chauveau and E. J. Marey addressed themselves to an experimental approach to the problem.

Chauveau was a professor of veterinary medicine in Lyons and an experimental physiologist with a particular interest in the relationship between heart sound and cardiac physiologic events. In 1856 Chauveau and his colleague²⁸

published a book describing their investigations of the cardiac movements and normal sounds.²⁰ Working at the School of Veterinary Medicine in Lyons they chose as experimental subject the horse whose slow heart rate facilitates direct observation.

Chapter IV of Chauveau and Falvre's text contains an analysis of the apical thrust, that is its timing and nature. In describing their predecessors' work, they cited the theories of Beau, Senai, Hunter, Beclard, Borelli, Bernard, Ponchappe, Hope, Falloon, and Hiffelstein, declaring that all were incorrect. In the view of Falvre and Chauveau "The apical thrust corresponds to the instantaneous change of the ventricular form and consistency at the beginning of systole [which is] transmitted to the entire anterior thoracic wall but felt only where there is no bony structure. Thus their conclusions were in agreement with those of Harvey and Laënnec."²¹

E. J. Marey, a young and brilliant Parisian physician, subscribed enthusiastically to a concept promulgated by Bernard, namely that experimental physiology is a key to the understanding of pathological phenomena. Prior to his collaboration with Chauveau, Marey had already devised a

sphygmograph which could record changes in the pressure of flowing blood. In his doctoral thesis²² he described studies of arterial pulse transmission and offered a physiological interpretation of the meaning of the dicrotic notch observed in the pressure waveform recorded in peripheral arteries.

The source of the idea of introducing a pressure recording apparatus into the heart to study the cardiac apex beat is not clear. It is possible that Chauveau and Marey were aware of Bernard's work and were encouraged by it to attempt pressure measurement in the cardiac cavities. Nor can we trace here the origin of the insight that tracings of intracardiac pressures could be related to cardiac physiologic events. Suffice it to note that it was generally accepted in French scientific circles in the early 1860s that systolic contractions caused increased intracavitary pressures and that changes in these pressures could be deduced from changes in appropriately obtained pressure tracings. To demonstrate the timing of the apical beat, it would suffice therefore to display its time course on a scale which also portrayed cyclic variations in ventricular and atrial pressures. To this at that time formidable problem in technique Chauveau and Marey directed their efforts.

In their first note on the cardiac apex beat (read before the Academy of Sciences)²³ they described a method of pressure recording using a catheter connected to a sphygmograph. As Chauveau and Marey acknowledged in a later report, the idea of connecting a system of levers to an instrument for continuous graphic recording must be credited to Karl Vierordt of Tübingen; this student of Ludwig evidently had constructed a sphygmograph to record the arterial pressure pulse. In Chauveau and Marey's arrangement flexible rubber ampoules were attached to both ends of the catheter, which was filled with water. One end of the catheter was introduced into a heart chamber; the ampoule at the external end was connected to the recording lever of the sphygmograph.²⁴ Another system of tubing with ampoules led from the cardiac apex to a similar device for recording of the apical beat. This approach failed "because of the resistance caused by inertia and friction of the long column of fluid within the tube"²⁵ and no doubt the elasticity of the rubber ampoules.

Therefore Chauveau and Marey had recourse to another transmitting device. They were familiar with a different technique for recording intraluminal

²⁰ *Nouvelle Recherches Experimentales sur les Mouvements et les Bruits Normaux du Cœur. Envisagés du Point de Vue de la Physiologie Médicale*. J. B. Baillière et Fils, Paris, 1856.

²¹ Chauveau and Falvre also deserve credit for the first experimental recording of pulmonary artery pressure as described in their book. They obtained this measurement with a needle in Chauveau's subsequent work he evidently never succeeded in advancing a catheter beyond the right ventricle.

²² *Recherches sur la Circulation du Sang*. Paris, 1859.

²³ Détermination graphique du rapport du choc du cœur avec les mouvements des oreillettes et des ventricles: Expérience faite à l'aide d'un appareil enregistreur (sphygmographe). *Compt. rend. des Séances de l'Académie de Science*. LIII, p. 622, October 7, 1861.

²⁴ See p. 273 in *Appareils et Expériences Cardlographiques. Démonstration nouvelle du mécanisme des mouvements du cœur par l'emploi des instruments enregistreurs à indications continues. Mémoires de l'Académie de Médecine*. 26, 768, 1863. The great care exercised by Chauveau and Marey in attributing priority to Vierordt for construction of the sphygmograph is noteworthy; elsewhere, with H. Zuckerkandl, I have advanced the view that recognition of priorities is one of the fundamental principles of the operating code of the scientist (*Studium Generale* 23, 941, 1970).

²⁵ See *Gazette Médicale de Paris*, May 18, 1861 and Chauveau and Marey, *Détermination graphique* (note 23).

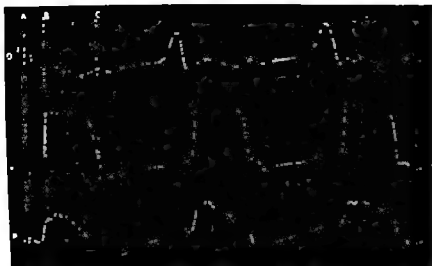


Fig. 7. Mechanism revealing of blood pressure I (the left) atrium and right ventricle and I (the apex) II.

Fig. 1. Simultaneous recordings (from bottom to top) of apical thrust and right ventricular and right atrial pulse pressures—Chauveau and Marey. See text and note 33.

pressures developed by Pierre Charles Buisson and described in his M.D. thesis.³⁰ This system consisted of a metal cup closed by an elastic membrane with a side connection to a long tube whose end led to a small metal funnel also closed by an elastic membrane. A stopcock allowed introduction of air into the system which could be used to record cyclic events.

Chauveau and Marey modified Buisson's system for their own purposes. Two long tubes bound together one longer than the other by approximately 12 cm and each closed at both extremities by a thin rubber balloon inflated with air were introduced through the jugular vein of an unanesthetized horse.³¹ They described at length the tracings they obtained and the timing of those pressure changes that were related to the impact of the apical heart

beat. They concluded that "it is unnecessary to emphasize further the significance of these tracings which seem to demonstrate in an irrefutable manner that the apical beat is an effect of ventricular systole" (Figure 1).

Several months later they published a second note³⁴ to answer a critique leveled by Beau. The latter tenaciously holding to his theory wondered how it was that the effect of auricular systole during ventricular diastole was not reflected in their tracings, an effect to which he attributed the beginning of the ventricular movement (diastole-systole) preceding its contraction. In their answer they stated that by refining

the sensitivity of the recording system and by increasing the amplitude and the speed of the tracing we were able to identify during the ventricular diastole all the accessory movement imparted to the blood in the [ventricular cavity] by the auricular systole (characterized by a small undulation of the ventricular tracing or even by the clapping of the valve).³⁵

According to a commendable tradition, the Academy of Sciences designated a committee to report on the two notes presented by Chauveau and Marey. Flourens, Rayer and Claude Bernard were members of the committee with Milne Edwards, who wrote their report. This document³⁶ was

³⁰ *Quelques recherches sur la circulation du sang à l'aide d'appareils enregistreur*. Faculté de Médecine de Paris, 1862. For another description of this device see the report by Milne Edwards (note 23).

³¹ *See Gazette Médicale de Paris*, May 18 1861 also *Détermination graphique* (note 29).

³² *Détermination graphique des rapports d'impact du cœur avec les mouvements des oreillettes et des ventricles (dernière note)*. *Compt. rend. des Séances de l'Académie des Sciences* LIV p. 32, Jan. 6, 1862.

³³ *Ibid.*

³⁴ *Op. cit.* (note 23).



The dual lumen rubber catheter constructed by Chauveau and Marey

Fig. 2 Double-lumen catheter: Chauveau and Marey's modification of Brissot's recording system. See text and notes 32 and 38.

published three months after the presentation of the second note and includes a description of the experiment on a horse which was carried out before the committee by Chauveau and Marey at the School of Veterinary Medicine of Alfort (near Paris). This report, constituting a model of presentation of a physiological problem, includes its historical background, describes in great detail the instruments used (sphygmograph and tubing) and the technique and discusses the results of the experiment. In their conclusion the committee accepted as undoubtedly proved correct the theory initially advanced by Marey.

B First systematic description and interpretation of intracardiac pressure recordings (1863)—Chauveau and Marey

In 1863 Chauveau and Marey presented what is perhaps their definitive report on the entire problem of intracardiac pressure recording and its interpretation.²² The report gives full details of the design, construction and operation of the recording system. The report is furthermore excellently illustrated and contains several reproductions of intraventricular pressure recordings. Analysis of their tracings demonstrated: (1) an interval of 0.1 sec from the beginning of atrial systole to the onset of ventricular systole, and (2) a 0.1-sec duration of atrial systole as compared with a 0.4 sec duration of ventricular systole. Chauveau and Marey also achieved the first recorded simultaneous measurement of pressures in the left ventricle and in the central aorta. They attempted to define the influence of left ventricular systole upon the contour of

the central aortic pressure curve. Moreover they were the first to refer to what later has come to be known as the isometric phase of left ventricular contraction. Their text goes into important detail regarding numerous other facets of cardiac pressure tracings in health and experimentally simulated disease which we cannot consider here. This work unquestionably is a milestone in the physiology of the heart.

In a separate publication²³ a now classical book published in the same year as their joint report, Marey set forth in detail the procedure utilized in these experiments describing the double-lumen catheter (Figure 2), the recording instruments and the tracings (Figure 3) with ample illustrations and legends. In this book he also described a special catheter to be introduced by the carotid artery route with which Chauveau and he secured pressure pulse tracings of the left ventricle (Figure 4). Moreover on the basis of carefully calibrated curves (and giving particular attention to temperature conditions in the heart, since blood pressure pulses are transmitted through a gaseous medium) Marey derived average figures for right atrial, right ventricular and left ventricular systolic pressures. These were respectively 2.5, 77.0 and 128.0 mm Hg—results which approximate figures subsequently reported in dog and in man.

Anticipating as it were the concerns of later physicians, in a footnote of his book Marey declared:

One can be reassured of the innocuity of this method by examining the horse who is scarcely disturbed while



Fig. 18.

Fig. 3 Simultaneous recordings of pulse pressures (from top to bottom) in right atrium, right ventricle, and left ventricle by Chauveau and Marey. See text and note 38.

²² "Appareil et Expériences Cardiographiques" (note 30).

²³ *Physiologie médicale de la Circulation et du Sang*. De lahay, Paris, 1863.



Fig. 3

Fig. 4 Special catheter designed by Chauveau and Marey to be introduced into the carotid artery for recording left ventricular pulse pressures (see text and note 39).



ents as usual. In only a few instances is the pulse rate slightly increased, especially at the time of the introduction of the catheter within the heart cavities.³⁹

In the Preface to his book Marey emphasized the importance of extending the limits of our senses in physical examination of patients and listed many cavities and canalicular systems which may be ex-

³⁹ *Ibid.*

⁴⁰ Fick, A. Ein neuer Blutwellenzeichner. *Arch. f. Physiologie* 1864 p. 563. For the proximal balloon connected to Marey's sphygmograph as a luteic, hollow "C"-shaped spring was substituted which coiled as air pressure increased within the tubing introduced into the heart.

⁴¹ It is noteworthy that Ludwig failed to refer to the ballistic galvanometer invented before the kymograph by Pouillet. Surprisingly also, he did not acknowledge the invention of the U manometer by Poiseuille.

⁴² Eine Verbesserung des Blutwellenzeichners. *Arch. f. die gesammte Physiologie* 30: 597 1883. The improvement consisted in the replacement of the "C"-shaped spring by a straight steel spring and an air transmitting tube with terminal elastic balloons.

⁴³ Ueber die Schwankungen des Blutdruckes in verschiedenen Abschnitten des Gefäss-Systems (Zum Theil nach Versuchen von D. Vuciff aus Belgrad in Russland). *Verhandl. der Würzburg. Physiol. Med. Gesellsch.*, N. B. IV 1873 p. 223. See also New York Academy of Medicine Library Board Pamphlet 570: *Arbeiten aus dem physiologischen Laboratorium Würzburger Hochschule 1872-1878*.

explored with catheters and specula in the human. One cannot but wonder: Why did not Chauveau and Marey extend their experimental method to the exploration of the circulation in man? Even a tentative answer to that question must take into consideration the overwhelming interest in the role of clinicopathologic studies in the advance of clinical medicine in general and of knowledge of cardiac diseases, in particular. This attitude was in stark contrast to the physiological approach to the improvement of diagnostic and therapeutic procedures which was to wait for passage of another three-quarters of a century.

In summary Chauveau and Marey were the first to systematically employ catheterization for the investigation of intracardiac pressures. The original features of their collaboration in the solution of the controversy regarding the origin and timing of the cardiac apex beat were to establish experimentally the exact timing of atrial and ventricular contractions, and their effects on both the movements of the heart and intracardiac and central aortic pressure recordings.

C. Critical discussion of the work of Chauveau and Marey on intracardiac blood pressure recording

From 1864 on until the end of the 19th century several physiologists in Germany and England contributed importantly to determination of the validity of the work referred to in the previous section.

1. *Adolph Fick (1864)* A physicist and physiologist, Adolph Fick was one of the first students and the prosector of Carl Ludwig in Zurich; he served in this capacity at the Institute of Physiology from 1852 until 1870, when he was promoted to professor of physiology in Würzburg, where he remained until the end of his active life. While in the Zurich Institute laboratory he developed a pressure recording device⁴⁴ based on a principle different from that of the sphygmograph. It involved a "C" spring whose movements were transmitted through a lever to a feather which graphically inscribed its motions on the kymograph invented by Ludwig.

This recording system was improved further after Fick moved to Würzburg.⁴⁵ With his model connected to a catheter filled with a bicarbonate solution with a lateral opening near the tip of the elastic tubing, Fick undertook a study of the form and level of blood pressure variations in the right and left cardiac cavities and published the results.⁴⁶

Fick's main contention in this report was that this recording system was superior in fidelity to that employed by Chauveau and Marey. He claimed to have discerned a number of artifacts in their published tracings and he criticized their methods for scaling the curves. His arguments however are of questionable validity as becomes manifest on considering several of his conclusions: (a) His method records blood pressure directly. (b) The atrial pressure curve is almost horizontal with minimal waving at a level of approximately 0 mm Hg during the whole of diastole. Is so gut wie constant and daher gleich null. (c) The idea that there can be as much as a 10 mm Hg pressure difference between the minimum and maximum atrial pressures is considered out of the question. (d) An elevation of pressure just before the onset of ventricular contraction is unrelated to atrial systole.

Striking as these distortions were. It was in the comparison of left ventricular and aortic peak levels during systole that Fick's technique was most flagrantly at fault. The flat peak systolic tracing of the left ventricle was much lower and of different contour from that of the corresponding and almost simultaneous curve in the aorta. Obviously the lateral opening of the catheter used to record left ventricular pressures was obstructed during systole. To explain this gross discrepancy and paradoxical finding Fick discoursed at length a surprising show of dialectic in a scientist who gave so many proofs of objectivity and scientific rigor.

In the same bound pamphlet which contains all the reports of physiologic work from 1872 to 1879 in the Würzburg Hochschule appeared a paper by Badoud a student of Fick which refers to the effect of spinal cord section on pulmonary arterial pressure variations as measured using the same recording system.⁴¹ The title was deceptive. In that the work described pressure recording in the right ventricle. Furthermore the conclusion that the pres-

sure drops in ventricular systole are due solely to transection of the medulla is questionable since the drop in systolic pressure in the carotid artery was of considerably greater magnitude.

2. Porte Rolleston Roy and Adams In the following years cardiac catheterization gained importance as an investigative technique. New manometric systems were developed and by the late 1880's the method was routinely used in physiological laboratories for measuring intracardiac pressures, particular attention being given to their relation to cardiac physiological events. There were however disputes concerning the possible influence of the catheter on signals transmitted from the cardiac cavity to the manometer.

Porter⁴² in his studies on the filling of the canine right heart cavities used a single- or a double-lumen rigid catheter made of silver-plated brass of 2.5 to 3 mm internal diameter and connected by rubber tubing of the same diameter to the manometric recording system. The total length of the connecting tubing was 30 to 40 cm. With this system he defined the pressure gradients between the right atrium and ventricle. In discussing his technique and the validity of his results Porter indicated that he was well aware of prior work by Rolleston and by Roy and Adams who both had rejected the cardiac catheter as a means of faithfully transmitting cardiac events to the manometer.

Rolleston⁴³ had emphasized the role of friction along the tube and connectors in causing artifacts but he admitted that the friction through a narrow tube of course is less in the case of a large animal such as a horse (the preferred subject in Chauveau and Marey's experiments) than in the instance of small animals such as dogs and rabbits. He concluded as follows. In these animals it seems absolutely essential to connect the heart with the recording system by some other method which is free from the objections above stated. "C. S. Roy and J. G. Adams"⁴⁴ were also critical of the technique introduced by Chauveau and Marey and of the validity of endocardial pressure tracings obtained with a catheter. Indeed they substituted for the catheter a short cannula introduced through the apex of the heart and connected its distal end to a piston manometer filled with oil.

Regarding the argument relating to friction, Porter had this to say:

The friction between the oil and the wall of the piston manometer is certainly great when the loss from this

⁴¹ Badoud, Emil. Über den Einfluss an den Harn auf den Druck in der Lungen Arterie. New York Academy of Medicine Library. Bound Pamphlet 570. *Arbeiten an den physiologischen Laboratorium Würzburger Hochschule 1872-1878*.

⁴² Porter, W. T. Researches in the filling of the heart. *J. Physiol.* 13: 513, 1877.

⁴³ Rolleston, H. D. Observations on the endocardial pressure curve. *J. Physiol.* 3: 35, 1887.

⁴⁴ 1861.

⁴⁵ Roy, C. S. and Adams, J. C. Heart beat and pulse wave. *Practitioner* 44: 83 and 44: 161, 1890.

source is added to the loss from friction in the catheter and connecting tube it may well be that the total effect introduces an error in the curve that the fault is with the catheter and tubes, I can by no means concede.²⁰

And he went on to discuss critical damping of which he approved.

D. Concluding remarks on the validity of intracardiac pressure recording

That controversy among physiologists who were concerned to obtain tracings that correctly and precisely reflected intracardiac and intravascular pressure variations was settled on a truly scientific basis shortly after the end of the 19th century. From 1903 on Otto Frank published a series of fundamental and classical papers in which he laid down the mathematical and theoretical basis of faithful reproduction in form, fine detail, magnitude, and timing of intravascular and intracardiac events.²¹

According to Frank's formulation and theoretical principles, recording devices must be constructed so as to have definite characteristics: (a) low effective mass of the manometer and mobility

of the fluid in the entire connecting and recording system, and (b) high coefficient of elasticity of the membranes (rubber or metal alloy). In addition, it was desirable that the optical recording system attached to the membrane provide high magnification. Construction of the system according to the first two requirements entailed that the natural frequency of the entire system extending from the membranes to the mouth of the tubing or cannula in the blood vessel (or cardiac cavity) would exceed, by a factor of at least four, the frequency of the fastest component of the inscribed curve (whose frequency can be as great as 25/sec). Frank postulated also that the quality of the recordings with regard to amplitude, phase relations, and fine detail might be improved by critical damping (almost to the point of but avoiding aperiodicity).

The development of pressure recording in man by means of intracardiac or intravascular catheterization witnessed substantial efforts in order to satisfy these fundamental principles.

3 MEASUREMENT OF BLOOD FLOW

We now turn to a new chapter in the early history of cardiac catheterization, the discovery of the quantity of blood ejected by the ventricles per unit of time—that is, the cardiac output.²²

A. Fick's principle

Adolph Fick, shortly after his promotion to professor of physiology at the Hochschule of Würzburg, presented a brief and now famous note before the Society of Physiology and Medicine of this university town in 1870. The note is entitled "Über die Messung des Blutquantums in den Herzventrikeln" (Figure 5). As it stands the title is misleading, since it suggests the measurement of the ventricular blood volume but in the first sentence of the report it becomes clear that the measurement refers to the volume of blood that is ejected with each systole.

In this note, about one printed page in length,²³ Fick states that the volume of blood flowing through the lungs per minute can be calculated by dividing the arteriovenous difference of oxygen or carbon dioxide—the amount of gas taken up or given off by a unit volume of flowing blood—into the total volume of the same gas taken up or given off by the lungs per minute. This determination re-

²⁰ *Op cit*.

²¹ Two of the most important of Otto Frank's papers are: Kritik der elastischen Manometer. *Z. Biol.* 44:445 1903 and Die Puls in den Arterien. *Z. Biol.* 46:441 1905. Additional references to other work by Frank (also published in *Z. Biol.*) and remarkable historical and critical analysis of pressure pulse recordings may be found in Carl J. Wiggner's text, *The Pressure Pulse in the Cardio-Vascular System* (Longmans, New York, 1928).

²² A first attempt to estimate the cardiac output must be attributed to Stephen Hales. At the end of the third experiment reported in *Haemasticks* (*op cit* note 20), whose purpose was measurement of the blood pressure in man, he bled the animal to death, then filled its left ventricle with melted beeswax. The volume of the solidified wax, reshaped by the normal resting heart rate gave a figure of 6 liters per minute for the cardiac output. This figure is obviously too small for an animal the size of a mare. I attempting to account for this, one notes that Hales ignored the probable reduction in the size of the cardiac cavity after exsanguination. Hales' reasoning also presupposed complete emptying of the ventricle during systole. It must have felt confident in his measurements, for table in *Haemasticks* gives estimates of the cardiac output of dogs, oxen, sheep, and men.

²³ *Phy. med. Ges. Würzburg* July 9 1870.

²⁴ An English translation of Fick's note appears in the chapter entitled "Output of the Heart" in the book edited by A. P. Fishman and H. W. Richards, *Circulation of the Blood—Arteries and Veins* (Chapter 2 Oxford University Press, New York, 1964 p. 96).

blood pressure in the pulmonary artery, aorta, or both for the determination of cardiac work.

Zuntz, formerly a student of Pflüger, started in 1886 and terminated in 1894 extensive physiologic studies of respiratory gas exchanges, heat production, ventilatory work, cardiac output and work, and systolic ejection velocity in horses at rest and during exercise. A detailed compendium of these studies forms the basis for a monumental monograph published in 1898.¹⁴ Chapter IX of this monograph supplies detailed information regarding the correct technique for measuring blood flow according to the Fick principle after introducing a ureteral catheter well oiled to prevent blood clotting, into the right atrium. Zuntz insisted on the essential procedure for validating results: simultaneous collection of expired air and of both mixed venous and arterial blood in equal amounts over a period of 1/2 to 2 minutes with the experimental subject in a stable state. In several tables he compared the gas exchange and blood respiratory quotients. In table LXXX he entered the blood flow values measured in all his experiments. It amounted to 29.55 liters per minute for an average weight of 347 kg, i.e. 84 cc/kg/min. The scientific quality of his work matched his scientific integrity. He acknowledged that as he started measuring

blood flow in horses in 1891 and reported his application of the Fick principle using the term "new method"¹⁵ he was not aware of Grehan and Quinquaud's priority. He also frequently referred to the study of cardiac pressure by Chauveau and Marey and using the time course of their curve for calculating blood systolic ejection velocity compared his calculations of blood velocity with those obtained previously in the horse by Chauveau et al.¹⁶ i.e. 68 cm/sec versus 57 cm/sec.

Zuntz indeed may be considered a real precursor in combined studies of respiratory gas transport and hemodynamics heralding a period 50 years hence when such combined studies were performed on a larger scale in normal and diseased man.

C Validation of the Fick principle in its original formulation

The validation of the Fick principle also had to wait for another half century since in his simplified formulation Fick had examined neither the exact significance of each of its terms nor the form of the equation to be derived from the principle, particularly in relation to the integration of the function over time and the variability of the values which its terms can assume. He also did not analyze the conditions under which the equation was applicable: (a) establishment of a so-called stable state; (b) cyclic variations of alveolar ventilation not in phase with cyclic variations of blood flow into the lung capillaries; and (c) the phase relation between flow at the site of collection of samples and the rate of collection.

To the extensive use of cardiac catheterization as a valid means of measuring blood flow in man must be attributed the impetus given to proper formulation and validation of the principle so simply enunciated by Fick 80 years previously.

¹⁴ Zuntz, N. und Hagemann, O. Untersuchungen über den Stoffwechsel des Pferdes bei Ruhe und Arbeit. *Landwirtschaftliche Jahrbücher. Zeitschrift für die wissenschaftliche Landwirtschaft und Archiv der königlich preussischen Landesökonomischen Collegiums* XXVII Band, Ergänzungsband III, Paul Parey publisher Berlin, 1898 pp. 1-438.

¹⁵ Zuntz, N. Eine neue Method zur Messung der circulirenden Blutmenge und das Arbeit des Herzens. *Ebdenda* LV: 1 (1893).

¹⁶ *Journal de Physiologie* III: 695 1860.

During the first quarter of this century cardiac catheterization suffered an eclipse from the physiologic scene. The causes of the eclipse lay mainly in the use of artificial preparations for the study of the dynamics of the circulation, in particular the heart-lung preparation so brilliantly studied by Starling. No one will deny the value of such studies. They favor accurate measurements and well controlled experiments and they allow a careful analysis of the mechanical and physiological factors involved in a complex system. But investigation in the intact animal has to my mind at least one superior merit, namely, that it provides a more secure foundation for the application of physiologic thinking and techniques to the study of man. This application is the theme of the next episode in the history of cardiac catheterization.

I FIRST CONTROLLED CATHETERIZATION OF THE RIGHT ATRIUM IN MAN

a self-experiment reported by Werner Forssmann in 1929³⁸ provides a dramatic prelude to the era of cardiac catheterization in man. Forssmann was at the time 25 years of age. He had recently graduated from medical school and was pursuing his surgical training at the clinic of Eberswalde, a small town near Berlin. With the boldness

of youth and against the advice of colleagues who had made an abortive prior attempt on him, he exposed one of his own left arm veins, introduced a ureteral catheter into his venous system and advanced it, under fluoroscopic control and using a mirror up to his right atrium. Forssmann allowed the catheter to remain in this position at least long enough that he could climb a few stairs to the X-ray department, where chest films were taken. During the entire experience there were, he reported, no untoward effects due to the presence of the catheter (Figure 6).

According to a historical note by Benatt³⁹ this first successful controlled right atrial catheterization had been preceded by an experiment on a human cadaver in the course of which Forssmann realized how easy it was to guide a ureteral catheter up the arm vein into the right atrium. One of his objects was to be able to use the technique as a means of rapidly administering undiluted emergency drugs directly into the heart. A first patient with purulent peritonitis was treated according to this procedure. Through the catheter she received 1 liter of glucose solution that contained adrenalin and strophanthin; the patient improved temporarily but then died. At autopsy the tip of the catheter was situated near the opening of the inferior vena cava. Forssmann also cited as additional objectives the possible use of the method for study of the heart and for diagnosis, which he did not further specify.

Attempts have been made to disclaim Forssmann a priority. In an addendum to his first report,⁴⁰ published a month later, he acknowledged that Unger had informed him of earlier work by Bleichröder's group. Indeed in 1912 Bleichröder, Unger and Loeb reported several successful passages of a catheter into arm or leg veins of patients with subsequent advancement of its tip into the axilla, or into the inferior vena cava.⁴¹ Some of these experiments were also made on themselves or their assistants. However these investigators did not control the exact location of the catheter tip by X-ray, which probably was not available at this time. Unger suggested however that during the course of an experiment in which Bleichröder was the subject, he mentioned a stabbing pain in the

³⁸ Forssmann, W. Die Sondierung des Rechten Herzens. *Klin. Wochenschr.* 8:7085, 1929.

³⁹ Benatt, A. J. Cardiac catheterization. A historical note. *Lancet* 746, 1949.

⁴⁰ In this addendum, published month later Forssmann quotes the letter he received from Professor E. Unger (*Klin. Wochenschr.* 8:2267, 1929).

⁴¹ Three separate notes were presented at a session of the Hufeland Medical Society in Berlin and reported (*Klin. Wochenschr.* 49:1503, 1911). Bleichröder, in opening statement, recounted his experience with passing catheters into the arteries and veins of dogs and into the veins of man, back in 1905; these experiments were not published in his note of 1911. Unger dealt with intraarterial therapy; he mentions that he had introduced catheters into the femoral veins of dogs more than 100 times, leaving the probe in situ for several hours without observing any blood clotting or other ill effect.

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DER SONDERUNG DES RECHTEN HERZEN

Von Dr. Werner Forssmann

Ans der II. Chirurgischen Abteilung des Kaiser-Wilhelms-Instituts für klinische Forschung, Berlin, led. v. H. SCHLÖSSER

Fig 6 Title page of the article describing Werner Forssmann's self-experiment. See text and note 59

chest, indicating that the tip of the catheter might have reached the right heart.

Other claims to priority do not even deserve mention.

In the light of what followed, Forssmann's experiments constituted remarkable progress. In one bold stroke he had demonstrated that a well known experimental technique, which he had applied under difficult but controlled conditions, could be extended to the study of man. This advance was potentially of paramount importance in the study of pathologic changes in the circulatory system since such changes could be reproduced with difficulty or not at all in animal experiments.

During the next 2 years, Forssmann, first in Sanerbruch Surgical Clinic where he found not support, but hostility and then in Eberswalde again, studied in dogs a new method of radiologic examination of the right side of the heart, in which radiopaque contrast medium was injected directly through the cardiac catheter into the right atrium. These studies were reported in 1931.⁶⁰ He also made at least six additional attempts to catheterize himself.⁶¹ In these studies he injected 40% Uroselectan through the catheter without securing adequate X-ray visualization (Figure 7)

⁶⁰ Forssmann, W. Über Kontrastdarstellung der Höhlen des lebenden rechten Herzens und der Lungenarterieller. *Munch. Med. Wchnschr.* 71, 439 (1931). His presentation at surgical meeting was the last one scheduled at the last session. It apparently did not arouse any interest in Germany for some time.

⁶¹ These additional self-experiments were mentioned to me by Forssmann when we first met in Heidelberg, in November 1952.

⁶² Göran Liljestrand, *In Le Prix Nobel en 1956*. Nobel Foundation, Stockholm, 1957.

To conclude this all-too-brief account, the comments by Professor Liljestrand at the presentation of the Nobel Prize in Medicine or Physiology in 1956 on the motivation and on the unhappy experience of the protagonist of these studies in man seem fitting:

It must have required firm conviction of the value of the method to induce self-experimentation of the kind carried out by Forssmann. His later disappointment must have been all the more bitter. It is true that the method was adopted in a few places—in Prague and in Lisbon—but on the whole Forssmann was not given the necessary support. He was, on the contrary, subjected in criticism of such exaggerated severity that it robbed him of any facilitation to continue. This criticism was based on an unsubstantiated belief in the danger of the intervention, thus affording proof that—even in our enlightened times—a valuable suggestion may remain unexploited on the grounds of a preconceived opinion. A contributory cause in this instance was presumably that Forssmann was working in *calculus* that did not clearly grasp the great value of his idea.⁶²

To be sure, the possible physiologic applications of Forssmann's experiments were rapidly realized

Herr Prof Dr. W. Forssmann
I feel very
Werner Forssmann
Aug 52

Ans der II. chirurgischen Abteilung des Kaiser-Wilhelms-Instituts für klinische Forschung, Berlin, led. v. H. SCHLÖSSER

Über Kontrastdarstellung der Höhlen des lebenden rechten Herzens und der Lungenarterieller

Von Dr. W. Forssmann, a. Assistent der II. chir. Abt. des Kaiser-Wilhelms-Instituts für klinische Forschung, Berlin, led. v. H. SCHLÖSSER

Sonderdruck aus der *Klinischen wöchentlichen Wochenschrift* 1931, Nr. 22, S. 439.

(L. F. Liljestrand, Verlag in Stockholm)

Fig 7 Title page of the article by Forssmann reporting his experience with injection of a radiopaque substance through a catheter into the right atrium. See text and note 63

In 1930 Jimenez Diaz and Cuenca described a successful attempt to introduce a ureteral catheter through a cannula into an arm vein of a moribund patient.⁶⁶ They were able to locate its tip in the right atrium and obtained X-ray evidence of its location. They suggested many possible developments of this procedure: therapeutic intracardiac injections in shock; sampling of mixed venous blood for de-termination and comparison with peripheral venous blood contents of glucose, lactic acid, and respiratory gases; injection of radiopaque substances for visualizing the pulmonary circulation; and even measuring auricular pressure by connecting the catheter with a manometric system. In a succeeding paper the same year⁶⁷ they reported studies on a few patients with cardiocirculatory insufficiency in which they compared the O₂ content in peripheral veins and atrial blood and in one case the arteriovenous difference.

⁶⁶ Jimenez Diaz, C. and Sanchez Cuenca, B. El Sondaje del Corazón Derecho. *Archivos de Cardiología y Hematología* 11 March 1930.

⁶⁷ Estudios de Insuficiencia Circulatoria. *Archivos de Cardiología Hematología* vol. 11 December 1930.

⁶⁸ Zur Bestimmung des zirkulatorischen gesamten Volumens nach dem Fickschen Prinzip (Gewinnung des rechten rechten Blutes mittels Herzsondierung). *Arch Med Wchsch* 77 1311 Aug 1 1930. In this column report Klein refers to presentation at the November 1929 meeting of the Verein Deutscher Ärzte in Prague, and another in April 1930 to the Wiesbaden Verh. Dtsche Ges. inner Medizin. H. gives many details regarding (1) the caliber of the ureteral catheter (he substituted a No. 6 for the No. 4 used initially by Forssmann, so to avoid suction when sampling blood); (2) the site of introduction, a subclavicular rather than an internal vein; (3) the position of the arm during introduction of the catheter—away from the thorax—to avoid entering the external jugular vein; and (4) the discarding of the initial blood withdrawn, before sampling and transfer under oil of the blood collected. 1/60% of 18 attempts the tip of the catheter was located in the heart under fluoroscopic control most times the atrium once in the inferior vena cava, and twice in the right ventricle (without untoward effect). In three cases the Fick principle was applied to the measurement of cardiac output. 1/ two patients with normal circulations (one with chronic diastolic sclerosis, another with neuritis of the corpus) based on O₂ data were 4.46 and 5.6 liters per minute. 1/ the third, a patient with leukemia and anemia, the cardiac output on the basis of both CO₂ and O₂ data was measured respectively 6.93 and 6.6 liters per minute. Klein did not appear to give attention to the stability of the metabolic state since not more than minutes lapsed between the introduction and the removal of the catheter. He reported no follow-up studies during the next

There were no further publications on catheterization from Jimenez Diaz's clinic. In the same year however O. Klein at the German University in Prague⁶⁸ reported the first measurements of cardiac output in several patients with various chronic systemic diseases; some terminal the measurements were obtained according to the Fick principle, with mixed venous blood samples secured from the right atrium. In 1932 Padilla Cossio and Berconsky of the University of Buenos Aires obtained mixed venous blood by the same method and by applying the Fick principle determined the cardiac output in two patients in order to compare the results with those obtained using the foreign gas (acetylene) method then in its early development.⁶⁹

Apart from the immediate and rapid development of pulmonary angiography in clinical medicine in Portugal,⁷⁰ Latin America,^{71, 72} and France,⁷³ use of catheterization for study of the circulation in nor-

year he published several papers on various medical subjects, including diabetes, typhoid, and pruritus.

⁶⁹ These authors published four articles in succession on the subject of cardiac catheterization: a first on technique (Sondeo del Corazón: técnica. *Semana Médica* 11 79 August 1931); a second on identification of the superior vena cava (Sondeo del Corazón. La vena cava superior y el borde derecho de la sombra de proyección de las grandes venas de la base. *Semana Médica* 2 391 Aug. 11 1931); third on determination of cardiac output (Sondeo del Corazón: Determinación del Volumen minuto circulatorio. *Semana Médica* 2:445 Aug. 18 1932); and a fourth, in which they described different types of peripheral circulation and listed the possible applications of the technique of cardiac catheterization (Sondeo del Corazón. Diversos tipos de circulación periférica. *Semana Médica* 645 Sept. 8 1932).

These three physicians were part-time assistants, pending their mornings at the Medical Clinic of the University of Buenos Aires. The circumstances under which they pursued their clinical investigations were obviously not conducive to more systematic study and extension of the technique a study which could have profited greatly from their close contact with Bernardo Houssay who was then interested in the physiology of the lungs.

⁷⁰ Ega, Moiz Lopo d. Carvalho, and Almeida Lima. La visualité des vaisseaux pulmonaires au rayons X par injection dans l'oreille droite de fortes solutions d'iodure de sodium. *Bull Acad de Med Paris* 105 627 April 14 1931.

⁷¹ Perez Ariz. A. El Sondaje del Corazón derecho su técnica y aplicación. *Rev de Med. Cir de la Habana* 36 491 July 31 1931.

⁷² Perez Ariz. A. Contribución al estudio de la anemia congenital del Corazón. *Rev de Med. Cir de la Habana* 36 657 Sept. 70 1931.

⁷³ Ameuille O. Ronneaux G. Huetet, V. Degrez and

mal and disease states was fragmentary and limited to exceptional instances which were of little im-

portance in furthering physiologic or patho-physiologic knowledge. Obviously many possibilities were envisaged but no systematic plan nor the proper scientific background and facilities for its wide-scale implementation, were then available.

Lemaire, J. M.. Remarques sur quelques cas d'artériographie pulmonaire chez l'homme vivant. *Bull. & Mém. Soc. Méd. d'Hop. de Paris* 60-729 1935. Severe criticism by the leading cardiologist in Paris was leveled at the authors, in spite of the fact that they had not encountered any mishaps. In the heated discussion which followed their presentation and the criticism, most participants favored discontinuing the procedure.

In short, the achievements of the early period of experimental studies were for the time being confined to a dead-letter office.

III THIRD PERIOD—1936-1945

The third of the periods I have recognized in the evolution of cardiac catheterization began quietly in 1936. The method was then still in eclipse in the experimental laboratory and its clinical use was sporadic and related to no definite programs of physiological inquiry. Subsequent developments which here can merely be outlined provide what is to my mind a provocative illustration of the fertile interaction among a general plan of scientific inquiry, technique, contingency and a fortunate group of investigators.

I PRELUDE

In 1932 Dickinson Richards then at the Columbia-Presbyterian Medical Center and I, chief resident of the Chest Service (Columbia University Division) at Bellevue Hospital in New York, agreed on a systematic and comprehensive examination of cardiopulmonary function, in normal and diseased man. Our working hypothesis had been propounded by Lawrence Henderson. The lungs, heart and circulation form a single system for the exchange of respiratory gases between the atmosphere and the tissues of the organism.¹⁹

In order to define the operation of this system (and thus validate the general concept) a number of

methods were required. Some of these methods were already available but others had to be devised "from scratch." From the beginning we realized that implementation of our general plan presupposed methods for determining respiratory gas concentrations in mixed venous and arterial blood and for measuring cardiac output. We succeeded in developing new techniques for studying various aspects of ventilatory and alveolo-respiratory function, but after several years we admitted failure of our attempts to equilibrate the CO_2 pressure in a rebreathing bag system with that in the mixed venous blood perfusing the lung capillaries. In patients with lung disease inhomogeneity of alveolar gas distribution and capillary blood perfusion made the required equilibration unattainable.²⁰ On the other hand insertion of a needle into a brachial artery could be accomplished routinely.

In 1936 we considered whether the technique of right heart catheterization might provide help in obtaining the mixed venous blood needed for determination of respiratory gas concentrations in accordance with our general plan. I arranged therefore to visit with Dr. Ameuille, one of my former teachers who had completed pulmonary angiography in 100 patients in his hospital in Paris.²¹ The protocols of these cases convinced me of the safety of the technique. Dick Richards and I in collaboration with Robert Darling then familiarized ourselves with the method first in canine experiments and eventually in a chimpanzee.²² Meanwhile George Wright, a former student of Carl Wiggers and then a resident on the Chest Service at Bellevue Hospital, offered to advance ureteral catheters under fluoroscopic control into the right atrium of human cadavers, in order to measure the distance between the catheter tip and a landmark on the anterior chest wall on lateral chest X-rays; this datum would be needed to establish a reference point for measuring intracardiac pressures.²³

DEVELOPMENTS FROM 1940 TO 1945

In 1940 our first attempt on a patient failed because the catheter was obstructed at the level of the atri-

For a succinct and comprehensive discussion of the development of this working hypothesis, see the text of the remarks made by D. W. Richards at the Respiration Dinner of the American Physiological Society at Chicago, April 17, 1957 (Lawrence Joseph Henderson, *The Physiologist*, vol. 1, 1957).

¹⁹ Richards, D. W., J. Courmand, A. and Bryan, N. A. Applicability of rebreathing method for determining mixed venous CO_2 in cases of chronic pulmonary disease. *J. Clin. Invest.* 14: 173, 1935.

²⁰ *Op. cit.* (note 73).

²¹ The chimpanzee experiment remained unpublished until it appeared in the *P&S Quarterly* (17: 15, Winter, 1977) under the authorship of D. W. Richards, who refreshed his recollection by referring to the experimental notebook of the Department of Pharmacology where the experiment had been performed.

²² Unpublished data which served as the basis for determining the zero pressure level in subsequent pressure recordings using saline manometer.

la.²³ Not long afterward however a new opportunity presented itself. Homer Smith, Professor of Physiology at the New York University College of Medicine, with his collaborators including Drs. William Goldring, Herbert Chasis, and Hilmer Ranges at the Hypertension Clinic of Bellevue Hospital was planning to measure cardiac output in hypertensive patients using a new model of the ballistocardiograph.²⁴ They had reached a stumbling block, however. To calibrate the ballistocardiograph curves they needed reliable independent values of cardiac output determined at the same time as the ballistocardiograph recording. For this purpose, application of the direct Fick principle

²³ The attempt was made at the Presbyterian Hospital in early 1940. It was not reported. The obstruction was due to large lymph nodes in the presence of metastatic carcinoma.

²⁴ The new ballistocardiograph was designed by Dugald E. S. Brown of the Department of Physiology, New York University College of Medicine. For description of this apparatus see the Appendix to subsequent report by Courmand et al. (note 82).

²⁵ Courmand, A., and Ranges, H. A., Catheterization of the right auricle in man. *Proc Soc Exper Biol & Med.* 46:462 1941.

²⁶ The initial results were presented at the Annual Meeting of the American Physiological Society in April 1941 held in Chicago. For published presentation of the results, see Courmand, A., Ranges, H. A., and Riley, R. L., Comparison of results of the normal ballistocardiograph and direct Fick method in measuring the cardiac output in man. *J Clin Invest.* 21:287 1942. The average figure for cardiac output were using the Fick method, 6.23 liters per minute and using the ballistocardiograph method 4.49 liters per minute.

²⁷ Richards, D. W., J. Courmand, A., Darling, R. C., and Gillespie, W. H. Pressure in the right auricle of man in normal subjects and in patients with congestive heart failure. *Trans A Am. Phy.* 56:218, 1941.

²⁸ Richards, D. W., J. Courmand, A., Darling, R. C., Gillespie, W. H. and Baldwin E. deP. Pressure of blood in the right auricle, in animals and in man: Under normal conditions and in right heart failure. *Am. J Physiol* 134:115 1942.

²⁹ A line of catheters was manufactured according to our specifications by the United States Catheter Company Glen Falls, New York.

³⁰ This cannula was manufactured by Becton and Dickinson, East Rutherford, New Jersey under the incorrect designation of "Courmand needle" the design of the needle was due to Richard L. Riley who followed suggestion by our research group.

³¹ Hamilton, W. F., Brewer D. and Brotman, J. Pressure pulse contours in the intact animals. I. Analytical description of a new high-frequency hypodermic manometer with illustrative curves of simultaneous arterial and intracardiac pressure. *Am J Physiol.* 107:4

appealed to the brilliant physiologist, and there was little need for persuasion.

In a first report published in 1941 in collaboration with Ranges the results of initial experiences were presented. The report emphasized (a) the necessity that the patient be metabolically stable (b) the requirement for simultaneous and prolonged blood and air sampling; (c) the value of the internal check provided by comparing results based on the application of the Fick principle to CO₂ and O₂ data (this resulted in rejection of at least half of the experiments) (d) the reproducibility of the results and (e) the acceptability to patients and innocuousness of the catheterization procedure.²⁵

The data on cardiac output were collected simultaneously according to the direct Fick and the ballistocardiograph procedures. The latter data were interpreted using Starr's formula. Comparison of the results indicated that the ballistocardiograph method gave values lower by 30% on the average than those obtained with the direct Fick technique in the 32 patients tested.²⁶ A few months later in 1941 Richards, Courmand, Darling and Gillespie published an initial report on simultaneous measurement of atrial and peripheral venous pressure with saline manometers in normal man and in patients with congestive heart failure.²⁷ In 1942 an exhaustive report on the same subject included our experience in animals as well as in man.²⁸

Between 1941 and 1945 several lines of technical advance were pursued.

(a) A new catheter was designed. Its construction by maintaining a desirable balance between flexibility and rigidity assured ready maneuverability. This probe was varnished with an unrettable radiopaque material and it had the minimal diameter compatible with facile sampling of blood using a slight degree of negative pressure. Finally because of its curvature near the tip this instrument could be readily manipulated both intravenously and in the heart.²⁹

(b) A cannula was also designed that facilitated insertion into the brachial or the femoral artery and prolonged cannulation of these vessels.³⁰

(c) With the assistance of Stanley Bradley our catheterization protocol was modified to include pressure recording with the Hamilton manometer.³¹

(d) Later a special mobile multiple recording apparatus was designed in collaboration with Lawson and Richard Bloomfield. It contained electrocardiograph, four fluid p

14446 P

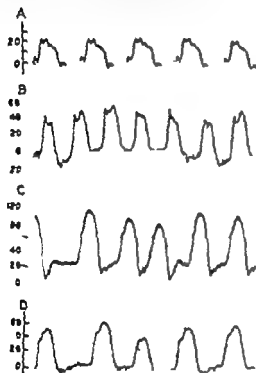
Recording of Right Heart Pressures in Man.

A. COURMAND, H. D. LAUBON, R. A. BLOOMFIELD, E. S. BREED, AND E. DE F. BALDWIN
 (Introduced by W. W. Palmer)

From the Department of Medicine College of Physicians and Surgeons, Columbia University;
 of Physiology, Medicine and Surgery New York University College of Medicine; and B Sroun
 Hospital New York City

Utilizing the right heart catheterization technique of Courmand and Ranges,¹ the cyclic pressure changes in the right auricle and right ventricle have been recorded with the Hamilton manometer² in 50 individuals.

Catheterization of the right auricle was performed as previously described.^{1,2} The



MS ORECC

FIG. 1.

All tracings as attached improve contrast in reproduction. Pressure mm Hg.

A. Right ventricular pressure pulse from normal young female. One respiratory cycle is shown.

B. Record from young male with extensive pulmonary thrombosis and normal heart. Cardiac output and arterial pressure normal. Note marked

increase in systolic pressure; large respiratory variation due to dyspnea. C. Postoperative sharp drop of systolic and diastolic levels associated with beginning of inspiration; artifacts on systolic peaks.

C. Patient with mitral stenosis and insufficiency and aortic insufficiency and aortic regurgitation in congestive failure. Note extreme elevation of systolic pressure in right ventricle. The high diastolic pressure corresponds to marked increase in right auricular and peripheral venous pressure. Note respiratory variation associated with dyspnea. Cardiac output subnormal and total blood volume about twice normal. Arterial pressure was 110/90.

D. Same patient after clinical improvement due to bed rest and digitalization.

patients experienced little or no discomfort during the procedure. The level of the catheter in the heart by lateral X-ray was taken as zero pressure. Introduction of the catheter with a slightly curved tip into the right ventricle under fluoroscopic control was not difficult as a rule and was signaled first by a rise above auricular pressure then by large oscillations at cardiac rate in the saline manometer connected to the catheter.

Addition of the long narrow catheter (No. 8 or 9) to the manometer system decreases the natural frequency. Frequencies obtained with this system have varied from about 25 to 50 vibrations per second. Although not ideal, this range has proved fairly adequate.

This investigation was carried on under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University with the collaboration of New York University. Additional support was provided by the Commonwealth Fund.

¹ Courmand, A., and Ranges, H., *Proc. Soc. Exp. Biol. Med.*, 1941, 48, 462.

² Hamilton, W. F., Brewer, O., and Brinson, J., *Am. J. Physiol.* 1934, 107, 437.

³ Courmand, A., Ranges, R. L., Breed, E. S., and Baldwin, E. de F. *Interim Report, O.S.R.D. Contract OEMC-107* 1943.

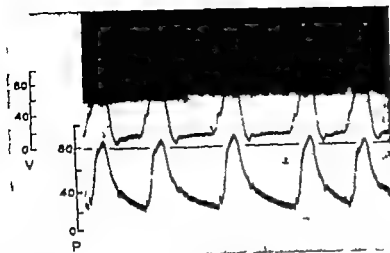


Fig. 8. Simultaneous tracings in the right ventricle (upper) and in the pulmonary artery (lower) in case of rheumatic valvulitis with hypertension in the lower circulation. Vol. () the identity of form and pressure of both tracings during the latter part of systole (b) the small arrhythmias occur from occlusion of the catheter in early systole and diastole presumably due to motion of the catheter in the heart.

manometers a fluid system for flushing a calibrating mercury manometer and an optical system for transmitting deflections of the manometers without parallax to the recording camera the total length of the optical bench was 6 feet.^{22,23}

These improvements in instrumentation were dictated by our concern for safety efficient teamwork adequate sampling of blood for analysis and reliability of intracardiac and intravascular pressure tracings.

A different kind of fundamental advance resulted from our decision to advance the catheter tip into

²² This mobile unit was constructed after discussion of, and improvement upon, our specifications by the Cambridge Instrument Company of New York City.

²³ The requirements for perfect recording of intracardiac pressures according to Otto Franck and Carl Wiggers were not achieved until many years later when an electronic recording system was devised by Laurent in Paris. Unfortunately in our early work we had not been able to interest electronic engineers in the design and fabrication of such device. The tracings obtained through a catheter show artifacts of variable extent, as result of the movement of the column of fluid in turn result of the beating of the heart. Moreover the best natural frequency of the entire recording system never exceeded 40. But with adequate damping, tracings were obtained which correctly reflected systolic and diastolic pressures, integrated mean pressure timing of partial deflections and general form.

Fig. 9 The first pressure pulse tracings recorded in the pulmonary artery of a patient from top to bottom, simultaneous tracings in the right ventricle and in the pulmonary artery. See text, legend and note 91

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2108, 2109, 2110, 2111, 2112, 2113, 2114, 2115, 2116, 2117, 2118, 2119, 2120, 2121, 2122, 2123, 2124, 2125, 2126, 2127, 2128, 2129, 2130, 2131, 2132, 2133, 2134, 2135, 2136, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2145, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160, 2161, 2162, 2163, 2164, 2165, 2166, 2167, 2168, 2169, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185, 2186, 2187, 2188, 2189, 2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197, 2198, 2199, 2200, 2201, 2202, 2203, 2204, 2205, 2206, 2207, 2208, 2209, 2210, 2211, 2212, 2213, 2214, 2215, 2216, 2217, 2218, 2219, 2220, 2221, 2222, 2223, 2224, 2225, 2226, 2227, 2228, 2229, 2230, 2231, 2232, 2233, 2234, 2235, 2236, 2237, 2238, 2239, 2240, 2241, 2242, 2243, 2244, 2245, 2246, 2247, 2248, 2249, 2250, 2251, 2252, 2253, 2254, 2255, 2256, 2257, 2258, 2259, 2260, 2261, 2262, 2263, 2264, 2265, 2266, 2267, 2268, 2269, 2270, 2271, 2272, 2273, 2274, 2275, 2276, 2277, 2278, 2279, 2280, 2281, 2282, 2283, 2284, 2285, 2286, 2287, 2288, 2289, 2290, 2291, 2292, 2293, 2294, 2295, 2296, 2297, 2298, 2299, 2300, 2301, 2302, 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2310, 2311, 2312, 2313, 2314, 2315, 2316, 2317, 2318, 2319, 2320, 2321, 2322, 2323, 2324, 2325, 2326, 2327, 2328, 2329, 2330, 2331, 2332, 2333, 2334, 2335, 2336, 2337, 2338, 2339, 2340, 2341, 2342, 2343, 2344, 2345, 2346, 2347, 2348, 2349, 2350, 2351, 2352, 2353, 2354, 2355, 2356, 2357, 2358, 2359, 2360, 2361, 2362, 2363, 2364, 2365, 2366, 2367, 2368, 2369, 2370, 2371, 2372, 2373, 2374, 2375, 2376, 2377, 2378, 2379, 2380, 2381, 2382, 2383, 2384, 2385, 2386, 2387, 2388, 2389, 2390, 2391, 2392, 2393, 2394, 2395, 2396, 2397, 2398, 2399, 2400, 2401, 2402, 2403, 2404, 2405, 2406, 2407, 2408, 2409, 2410, 2411, 2412, 2413, 2414, 2415, 2416, 2417, 2418, 2419, 2420, 2421, 2422, 2423, 2424, 2425, 2426, 2427, 2428, 2429, 2430, 2431, 2432, 2433, 2434, 2435, 2436, 2437, 2438, 2439, 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2606, 2607, 2608, 2609, 2610, 2611, 2612, 2613, 2614, 2615, 2616, 2617, 2618, 2619, 2620, 2621, 2622, 2623, 2624, 2625, 2626, 2627, 2628, 2629, 2630, 2631, 2632, 2633, 2634, 2635, 2636, 2637, 2638, 2639, 2640, 2641, 2642, 2643, 2644, 2645, 2646, 2647, 2648, 2649, 2650, 2651, 2652, 2653, 2654, 2655, 2656, 2657, 2658, 2659, 2660, 2661, 2662, 2663, 2664, 2665, 2666, 2667, 2668, 2669, 2670, 2671, 2672, 2673, 2674, 2675, 2676, 2677, 2678, 2679, 2680, 2681, 2682, 2683, 2684, 2685, 2686, 2687, 2688, 2689, 2690, 2691, 2692, 2693, 2694, 2695, 2696, 2697, 2698, 2699, 2700, 2701, 2702, 2703, 2704, 2705, 2706, 2707, 2708, 2709, 2710, 2711, 2712, 2713, 2714, 2715, 2716, 2717, 2718, 2719, 2720, 2721, 2722, 2723, 2724, 2725, 2726, 2727, 2728, 2729, 2730, 2731, 2732, 2733, 2734, 2735, 2736, 2737, 2738, 2739, 2740, 2741, 2742, 2743, 2744, 2745, 2746, 2747, 2748, 2749, 2750, 2751, 2752, 2753, 2754, 2755, 2756, 2757, 2758, 2759, 2760, 2761, 2762, 2763, 2764, 2765, 2766, 2767, 2768, 2769, 2770, 2771, 2772, 2773, 2774, 2775, 2776, 2777, 2778, 2779, 2780, 2781, 2782, 2783, 2784, 2785, 2786, 2787, 2788, 2789, 2790, 2791, 2792, 2793, 2794, 2795, 2796, 2797, 2798, 2799, 2800, 2801, 2802, 2803, 2804, 2805, 2806, 2807, 2808, 2809, 2810, 2811, 2812, 2813, 2814, 2815, 2816, 2817, 2818, 2819, 2820, 2821, 2822, 2823, 2824, 2825, 2826, 2827, 2828, 2829, 2830, 2831, 2832, 2833, 2834, 2835, 2836, 2837, 2838, 2839, 2840, 2841, 2842, 2843, 2844, 2845, 2846, 2847, 2848, 2849, 2850, 2851, 2852, 2853, 2854, 2855, 2856, 2857, 2858, 2859, 2860, 2861, 2862, 2863, 2864, 2865, 2866, 2867, 2868, 2869, 2870, 2871, 2872, 2873, 2874, 2875, 2876, 2877, 2878, 2879, 2880, 2881, 2882, 2883, 2884, 2885, 2886, 2887, 2888, 2889, 2890, 2891, 2892, 2893, 2894, 2895, 2896, 2897, 2898, 2899, 2900, 2901, 2902, 2903, 2904, 2905, 2906, 2907, 2908, 2909, 2910, 2911, 2912, 2913, 2914, 2915, 2916, 2917, 2918, 2919, 2920, 2921, 2922, 2923, 2924, 2925, 2926, 2927, 2928, 2929, 2930, 2931, 2932, 2933, 2934, 2935, 2936, 2937, 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3104, 3105, 3106, 3107, 3108, 3109, 3110, 3111, 3112, 3113, 3114, 3115, 3116, 3117, 3118, 3119, 3120, 3121, 3122, 3123, 3124, 3125, 3126, 3127, 3128, 3129, 3130, 3131, 3132, 3133, 3134, 3135, 3136, 3137, 3138, 3139, 3140, 3141, 3142, 3143, 3144, 3145, 3146, 3147, 3148, 3149, 3150, 3151, 3152, 3153, 3154, 3155, 3156, 3157, 3158, 3159, 3160, 3161, 3162, 3163, 3164, 3165, 3166, 3167, 3168, 3169, 3170, 3171, 3172, 3173, 3174, 3175, 3176, 3177, 3178, 3179, 3180, 3181, 3182, 3183, 3184, 3185, 3186, 3187, 3188, 3189, 3190, 3191, 3192, 3193, 3194, 3195, 3196, 3197, 3198, 3199, 3200, 3201, 3202, 3203, 3204, 3205, 3206, 3207, 3208, 3209, 3210, 3211, 3212, 3213, 3214, 3215, 3216, 3217, 3218, 3219, 3220, 3221, 3222, 3223, 3224, 3225, 3226, 3227, 3228, 3229, 3230, 3231, 3232, 3233, 3234, 3235, 3236, 3237, 3238, 3239, 3240, 3241, 3242, 3243, 3244, 3245, 3246, 3247, 3248, 3249, 3250, 3251, 3252, 3253, 3254, 3255, 3256, 3257, 3258, 3259, 3260, 3261, 3262, 3263, 3264, 3265, 3266, 3267, 3268, 3269, 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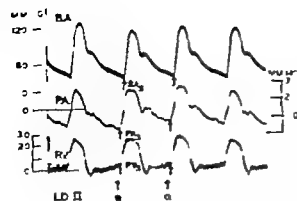


Fig 11 Simultaneous recordings of pulse pressures (from top to bottom) in femoral artery, pulmonary artery and right ventricle and electrocardiogram. These recordings were made in a man with normal circulation using the mobile multiple recording apparatus. See text and note 88.

the right ventricle (Figure 8). This was done in 1942.^{88,89} Two years later the catheter was advanced into the pulmonary artery (Figure 9) providing thereby the real key to studies of the pulmonary circulation in health and disease. This last development and our repeated investigations of the problem of the optimal location for obtaining truly representative samples of mixed venous blood

in construction of double lumen catheters (Figure 10).⁹⁰ This made it possible to secure simultaneous blood samples and pressure recordings in the right atrium and ventricle (Figure 11) or in the right ventricle and pulmonary artery.

During the same period, i.e. 1942-1944, we studied various forms of shock, analyzing their physiologic mechanisms and evaluating the effects of different therapies. Over a period of 4 years a team of several individuals studied 103 cases in all. In each of these cases, over at least a several-hour period, cardiac output, blood volume, intracardiac and intraarterial blood pressures, respiratory gas exchanges, blood gases and pH, and in some cases, renal blood flow were repeatedly measured. The results of this clinical investigation were presented in a series of reports to the Committee on Medical Research of the Office of Emergency Management, an arm of the Office of Scientific Research and Development; these results were also published in successive papers⁹¹ and given elegant form in the Harvey Lecture of 1944 by D. W. Richards (Figure 12).⁹²

Meanwhile, we also investigated following similar protocol the circulatory effects of pressure

breathing and phlebotomy in normal subjects and of bilateral sympathectomy in those with systemic hypertension. We also had the opportunity to study a patient with systemic hypertension before and during the evolution of cardiac failure. Cardiac failure of two other types—that due to rheumatic heart disease and that associated with cor pulmonale—was compared on the basis of observations in respectively seven and six subjects. These studies were presented at a symposium on cardiac output, organized by the American Physiological Society in Philadelphia in 1944 and published in the *Federation Proceedings*⁹³ in 1945 (Figures 13-17).

During this period original contributions marking

⁸⁸ Semi-annual report of work under Contract 107 with O.E.M. (C.M.R.) for the period from September 1942 to March 1943; see pp. 35-58 and chart 13 (National Research Council, Washington, D.C.).

⁸⁹ Coermann A., Larson, H. D., Bloomfield R. A., Breed E. S., and Baldwin, E. deF. Recording of right heart pressures in man. *Proc Soc Exper Biol & Med* 55:34, 1944.

⁹⁰ The first recorded pressure tracings in the pulmonary artery of a patient with rheumatic heart disease in failure (figure 11) report published in *Proc Soc Exper Biol & Med* 1945 (note 91).

⁹¹ Coermann A., Bloomfield R. A., and Larson, H. D. Double lumen catheter for intravenous and intracardiac blood sampling and pressure recording. *Proc Soc Exper Biol & Med* 61:73, 1945.

⁹² See Coermann A., Riley R. L., Bradley S. E., Breed, E. S., Noble R. P., Larson H. D., Gregersen M. I., and Richards, D. W. Studies of the circulation in chemical shock. *Surgery* 13:964, 1943; Coermann A. Studies of the circulation in shock. *Proc. Radolf Virchow Med Soc.* 151, 1943; Larson, H. D., Bradley S. E., and Coermann, A. The renal circulation in shock. *J Clin Invest* 23:381, 1944; Coermann A., Noble, R. P., Breed, E. S., Larson, H. D., Baldwin, E. deF., Pinchot, O. B., and Richards, D. W. Chemical, clinical and immunological studies on the products of human plasma fractionation. VIII. Clinical use of concentrated human serum albumin in shock, and comparison with whole blood and with rapid saline infusion. *J Clin Invest* 23:491, 1944; and Richards, D. W. J., and Coermann A. Circulation in shock. Mechanical and vasomotor factors. *Trans A Am Phys* 58:111, 1944.

⁹³ The Circulation in Traumatic Shock in Man. *The Harvey Lecture Series XXXIX*, 217-253, 1943-1944. Chapter XXVII of *Advances in Military Medicine* (Little Brown, and Co. Boston, 1948), which reported all the projects completed under the auspices of O.E.M. (C.M.R.) during World War II contains section on the clinical aspects of shock which was written by D. W. Richards (pp. 335-341).

⁹⁴ Coermann A. Chaperon: Symposium on cardiac output. *Fed Proc* 4:183, 1945.

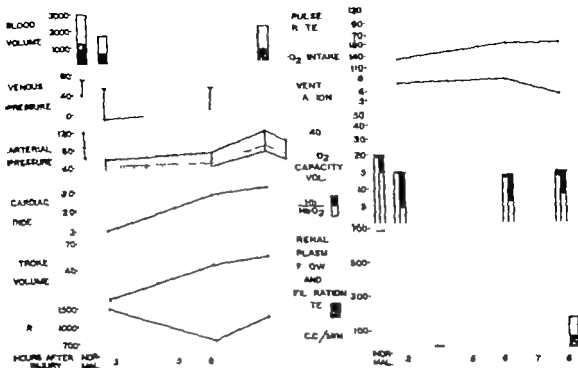


Fig 12 Illustration showing all data obtained over an 8-hour period from a patient with traumatic shock. On the horizontal time scale the solid bar represents the duration

of blood transfusion. For further details see text and note 94

the extension of cardiac catheterization into new fields were made by former collaborators or by investigators trained in our laboratory. Stanley Bradley, a member of our initial team, after his transfer to Boston, introduced as an investigative tool catheterization of the renal⁹⁴ and hepatic⁹⁵ versus James Warren in Atlanta,⁹⁶ and Eleanor Baldwin and Robert Noble⁹⁷ at the Columbia-

TABLE 1

Effect on cardiac output of continuous positive pressure breathing (30 mm Hg) in four normal subjects at sea level

PATIENT	RESPIRATORY PRESSURE mm. Hg	OXYGEN UPTAKE ml./min.	CARDIAC OUTPUT liters
L. K.	0	188	4.96
	+30	(187)	3.98
	0	187	5.36
G. T.	0	123	3.36
	+30	(122)	2.34
	0	126	4.01
G. G.	0	143	4.67
	+30	(137)	3.77
	0	143	3.46
E. R.	0	126	3.23
	+30	(126)	2.36
	0	124	2.94

Intervals between respiratory measurements about 30 minutes.

Fig 13-17 Tables published in *Federation Proceedings*, 1945. See text and note 95

Fig 13 Effects on cardiac output of continuous pressure breathing

⁹⁴ Catheterization of the renal vein with an extra-length (four-foot) radiopaque catheter by Bradley and Curry is described in Appendix G (p. 219) of *Hypertension and Hypertension Data* by William Goldring and Herbert Chases (The Commonwealth Fund, New York, 1944). The authors indicated that J. V. Warren, A. J. Merrill and E. S. Braunson had been engaged in similar studies to determine the percentage withdrawal of sodium para-amino hippuric acid.

⁹⁵ Bradley, S. E., Ingelfinger, F. J., Bradley, G. P. and Curry, J. J. The estimation of hepatic blood flow in man. *J. Clin. Invest.* 4: 390-397, 1945.

⁹⁶ Braunson, E. S., Weems, H. S. and Warren, J. V. Atrial septal defect. Study of hemodynamics by the technique of right heart catheterization. *Am. J. Med. Sci.* 210: 480, 1945.

⁹⁷ Bald, in E. deF. Moore, L. V. and Noble, R. P. The demonstration of ventricular septal defect by means of right heart catheterization. *Am. Heart J.* 32: 152, 1946.

investigators and none other to my knowledge has had the auspices of a perfect and lasting collaboration between two investigators with complementary qualities

In retrospect it would appear that the following were among the considerations which helped to make possible the successful extension to man of the technique pioneered by Claude Bernard. (a) The aim was always to improve diagnostic methods and to establish treatment of disease processes on a rational basis. (b) The means included an assemblage of valid techniques, used with awareness of past and present physiological developments. (c)

Colleagues were inspired with confidence as to the validity and reliability of the results and in the patients under our care as to the safety of the method. (d) We were able to take advantage of contingencies and to eliminate unnecessary risks. (e) Strict discipline was enforced in applying the technique in developing efficient team work, and in analyzing physiologic and pathologic conditions.

In a word we were fortunate enough to achieve in some measure the fundamental association of experimental and clinical medicine of which Claude Bernard dreamed.

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Studies in asymptomatic primary hyperlipidaemia

I Types of hyperlipoproteinaemias and serum lipoprotein concentrations, compositions and interrelations

By Anders G Olsson and Lars A. Carlson

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Hälsokontrollen Metropol Stockholm, Sweden.*

Studies in Asymptomatic Primary Hyperlipidaemia

I Types of hyperlipoproteinaemias and serum lipoprotein concentrations, compositions and interrelations

By
Anders G. Olsson and Lars A. Carlson

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Abbreviations	IHD = ischaemic heart disease
	HL = hyperlipidaemia
	LP = lipoprotein
	HLP = hyperlipoproteinaemia
	TG = triglycerides
	VLDL = very low density LP
	LDL = low density LP
	HDL = high density LP

INTRODUCTION

Atherosclerosis is a chronic disease developing over decades. Already during childhood and the first decades of life raised atherosclerotic lesions have been found in autopsy studies (53). The sudden clinical manifestations of atherosclerosis, such as myocardial infarction as well as those more slowly developing such as intermittent claudication, are merely the final clinical consequence of this long-lasting pathological process of the vessel wall.

Epidemiological studies during the 1950ies and 1960ies have revealed a number of "risk factors" for clinical manifestations of atherosclerosis, for instance ischaemic heart disease (IHD). The presence of such risk factors in an individual is associated with an increased risk for the development of IHD. Important risk factors for IHD are hyperlipidaemia (HL), glucose intolerance, cigarette smoking and hypertension.

With regard to HL retrospective (3, 11, 42) and prospective (16, 30, 54) studies have demonstrated that elevation of serum cholesterol (30, 42, 54) and triglyceride (TG) (3, 11, 16) concentrations are of importance in the development of IHD. The serum cholesterol and TG are contained in three different major lipoprotein (LP) classes but only scant information on the role played by these different LPs in the development of IHD is available. Considering the LPs in HL, Fredrickson et al (24) introduced a system in which in its modified form (4) describes six different types of hyperlipoproteinaemia (HLP). The importance of these types of HLP in atherosclerosis and its manifestations is not well defined. Retrospective studies have shown that elevation of both very low density LP (VLDL) and low density LP (LDL) (types IV and II B HLP) as well as decreases of high density LP (HDL) (II, 17B, 40) are associated with IHD but prospective studies on the role of the different types of HLP in the development of IHD are lacking. Retrospective as well as prospective studies have several disadvantages in studying the relation between HLP and IHD. In retrospec-

tive studies the clinical event in itself might have influenced the LP concentrations as well as other risk factors. Also many subjects have died during the acute phase of IHD. Prospective studies on the other hand are time consuming, require large numbers of subjects and would be quite costly if quantitative LP analyses were to be performed.

Against this background we considered it of practical and theoretical value to investigate newly discovered hyperlipidaemic but otherwise healthy subjects for the prevalence of atherosclerosis in the coronary arteries and the arteries of the lower limb in relation to different types of HLP. It is not possible today to estimate the degree of atherosclerosis by non-invasive techniques. Therefore we have chosen to investigate persons with HLP using methods which might disclose ischaemia and changes in arterial flow induced by atherosclerosis, the results serving as probable early signs of atherosclerosis. Furthermore by searching for metabolic abnormalities in such asymptomatic subjects in whom arterial disease has not yet given rise to symptoms, etiological mechanisms behind the HLP might be better elucidated than by studying patients with HLP and symptoms of atherosclerosis.

We have called the concept of tracing subclinical signs of a disease process in the presence of a certain characteristic - e.g. a risk factor such as HLP - before the appearance of the potential clinical event an *interceptive study*. The early detection of HLP also enabled us to take primary preventive measures i.e. to try to interrupt the process of atherosclerotic disease. We have undertaken an interceptive study in order to describe the presence of probable early signs of atherosclerosis in different types of HLP. The occurrence of these signs has also been determined in a control group with non-elevated concentrations of serum cholesterol and TG. HL has been defined in terms of LP concentrations and the HL hence is called HLP.

The purpose of this paper is to describe first the

serum lipids of the sample from which the HLP subjects were drawn ("The Metropol sample") and the formation of the interspective HLP and control groups. After that the frequency of different types of HLP and the LP concentrations, compositions and interrelationships in HLP will be reported.

"THE METROPOL SAMPLE"

PROCEDURE AND METHODS

"The Metropol Health Control Company" (Hälsokontrollen Metropol) Stockholm (Head Sture Helander, M. D.) in the following called Metropol, annually performs regular health examinations of around 15 000 people living in the Stockholm metropolitan area. The subjects coming to Metropol are employees of around 400 different companies which offer the health examination annually free of charge. The examination is voluntary and takes place during working hours. The result of the examination is only available to the subject and the doctor responsible. Inquiry was made to the staff manager of 25 randomly selected companies utilizing Metropol regarding the participation rate. This was 95 ± 2 (SEM) per cent (range 75–100 per cent). At Metropol, all subjects gave their medical history and received a complete physical examination by a physician. A resting ECG was recorded. Urine was analysed for the presence of glucose and protein and bacteria (BM test 4 Boehringer-Mannheim). Haemoglobin and ESR were also determined. Blood was taken every third year for serum cholesterol (6) and TG (31) analyses by venipuncture in the sitting position with as light venostasis as possible. For practical reasons blood sampling had to take place also in the afternoon and therefore both fasting and non-fasting subjects were examined. Clotting proceeded at room temperature for 1–2 hours, serum was recovered after slow speed centrifugation and stored at -20°C . The samples from one week, usually around 150, were analysed the next week at King Gustaf V Research Institute on one week day by the same technicians throughout the study on an Autoanalyzer Model 1 (Technicon Corporation). Cholesterol and triolein (reagent grade) from Sigma Chemicals were used as standards. Two control standard sera with defined and different concentrations (deep frozen pooled samples, not older than 6 months) were included in all runs. All

determinations of unknown samples were corrected according to the deviation of the standard serum. Standard sera were not allowed to deviate from each other by more than 3 per cent within each run. The error of the methods of the determination of cholesterol and TG have been around 4 and 5 per cent respectively.

When not otherwise stated, statistical treatment was performed according to Snedecor (52).

RESULTS

Serum lipids were analysed in 20 133 samples from 11 898 males and 8 234 females from 77 October 1970 till 18 March, 1974.

The distributions of serum cholesterol and TG of all men and women aged 41–45 years are given in Figures 1 and 2 and Table 1 to exemplify the "Metropol lipid pattern". The distribution curves clearly demonstrate the difference between the response of serum cholesterol and TG to the non-fasting state. In both sexes, the approximately normal distribution of the cholesterol concentrations were very similar in the fasting and non-fasting states. Mean values did not differ in men, but non-fasting women had total serum cholesterol slightly higher than fasting ($p < 0.05$).

On the other hand the skewed distribution of serum TG (10) was still more pronounced in the non-fasting state. Both the distribution curves of the TG values themselves and of their logarithms were displaced to the right (Fig. 1 and 2) in non-fasters and the mean serum TG was increased by around 50 and 30 per cent in men and women in non-fasting as compared to fasting state.

Although the distribution curves of total serum cholesterol in the two sexes were similar and approximately normal, the mean value in fasting men was 3 per cent higher ($p < 0.005$) than in fasting women. No difference was seen in mean

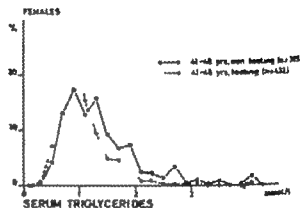
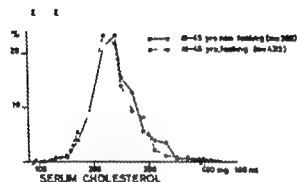
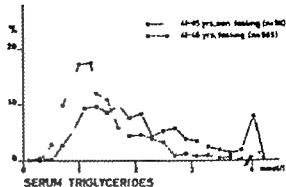
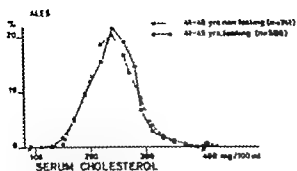


Fig 1 Serum cholesterol and TG in non-fasting and fasting males and females aged 41–45 years in the M tropol sample.

Table 1 Mean fasting and non-fasting serum cholesterol and TG concentrations in 41–45 year old males and females : Metropol

	MEN		WOMEN	
	Fasting 305	Non-fasting 714	Fasting 432	Non-fasting 365
Cholesterol, mg/100 ml				
Mean	239	238	232	239
SE	2	1	2	2
% > 340 mg/100 ml	1.8	1.6	1.6	1.6
% > 400 mg/100 ml	.6	1	.2	.2
TG mmol/l				
Mean	1.50	2.24	1.07	1.43
SE	0.04	0.05	.03	.06
% > 3.40 mmol/l	3.8	13.0	1.2	2.7
% > 4.00 mmol/l	2.3	8.1	1.2	1.6
Mean ¹	1.31	1.94	.97	1.24

¹ Calculated on logarithmic values and reconverted.

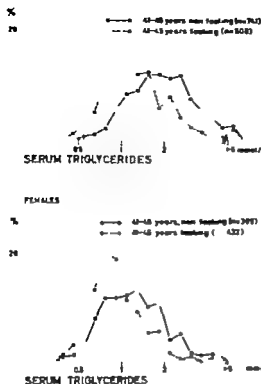


Fig. 2. Log TG in non-fasting and fasting males and females aged 41-45 years in the Metropoli sample.

serum cholesterol concentration between non-fasting men and women. The proportion of men and women with fasting serum cholesterol above 340 mg/100 ml was of similar magnitude. Mean serum TG concentration was 40 percent higher in fasting males than in females. The difference between the sexes is also demonstrated by the fact that three times more men than women had fasting TG concentrations above 4 mmol/l.

DISCUSSION

As seen before in this city (18) and elsewhere (e. g. 2) males and females 41-45 years have similar serum cholesterol concentrations while that of fasting serum TG is higher in males (18). In the non-fasting state the same sex relations existed for the serum lipids. The non-fasting state had exerted little or no influence on cholesterol values while the TG had increased considerably. For a long

time it has been a well known fact that alimentary lipaemia gives rise to higher serum TG concentrations. If the increase in serum TG of 0.7 mmol/l or 60 mg/100 ml was due only to chylomicrons it can be calculated from the known composition of this LP class that the cholesterol content of these chylomicrons would be about 1-2 mg/100 ml, value which is of similar magnitude as the observed differences in total serum cholesterol between fasters and non-fasters.

It is concluded that the state of fasting of subjects in lipid screening on a health control basis is of no practical importance as far as the serum cholesterol concentration is concerned. However as serum TG is profoundly altered in the non-fasting state non-fasting individuals displaying hypertriglyceridaemia should be re-investigated in the fasting state.

Ten years before the present study serum lipids were analysed at another health control centre in Stockholm (18). In this study The Stockholm Prospective Study the mean values (\pm SE) were for cholesterol (Sperry Webb method equivalent see ref. 18 p. 4) in males and females 40-44 years 37 ± 3 and 48 ± 3 mg/100 ml. The corresponding figures for fasting serum TG were 1.58 ± 0.04 and 1.07 ± 0.03 mmol/l. In the present study mean serum cholesterol values were thus 10-20 mg/100 ml lower than in the Stockholm Prospective Study while serum TG were almost identical. The percentage number of 40-45 year old males having serum cholesterol concentrations above 340 mg/100 ml in Stockholm prospective ($n=289$) and interspective ($n=505$) study were 2.7 and 1.8 respectively. For women the corresponding figures were 3.0 ($n=269$) and 1.6 per cent ($n=432$) respectively. The differences were not significant (22). In the same age group the percentage fasting serum TG of 3.00 mmol/l and above in Stockholm prospective and interspective studies were 5.5 and 5.7 per cent (n.s.) in men and 0.4 and 1.6 per cent (n.s.) in women.

It should be kept in mind that these two studies were not designed for the comparison of serum lipid values in 1961/62 and 1970-1974. The two different cholesterol methods have, however been repeatedly checked and standardized against the Sperry-Webb method at King Gustaf V Research Institute and supervised by one of us

(L.A.C.) The cholesterol method used in this study gives close to identical values with the Sperry Webb method, while the values from the prospective study can be transformed by a regression equation to be nearly identical to Sperry Webb (18). It is thus not unlikely that the lower mean cholesterol values observed in this study reflect a true decrease in serum cholesterol in subjects attending health control centres linked to their employment. The reason for this may at least partly be changed dietary habits. The total amount of fat available for consumption per capita in Sweden decreased between 1965 and 1973 from 123 g to 118 g daily (35). The percentage fat

calories in the food decreased from 40.4 to 38.6 per cent. On the other hand the consumption of linoleic acid increased both in relative and absolute terms. One possible explanation to the observed decrease in serum cholesterol may therefore be that the consumption of saturated fats has decreased and that of polyunsaturated fats has increased during the last decade.

The method for serum TG used here agrees well with Carlson's method (12) which was used in the Stockholm Prospective Study. Therefore we believe that there have not been any significant changes in the serum TG levels since 1961/62.

FORMATION OF THE INTERSPECTIVE SAMPLE

MATERIAL AND METHODS

Procedure

The screened subjects were grouped into different categories regarding the serum cholesterol and TG concentrations. Those with serum cholesterol above approximately 350 mg/100 ml and fasting serum TG above 3.5 mmol/l were with the exceptions noted below referred to the Lipid Unit at the Department of Internal Medicine Karolinska Sjukhuset for further examination and treatment. If in a non-fasting subject it was unclear whether an elevated serum TG value was due to alimentary lipemia only the subject was called back for a fasting sample at Metropoli. The above procedure was then applied.

At the first visit to the Lipid Unit, fasting blood was taken for serum lipid determination and LP analysis. The interval between the fasting blood samples at Metropoli and the Lipid Unit was 107 ± 3 (SEM) days. The same procedure was followed for the blood samples from the Lipid Unit as for the samples from Metropoli with regard to serum lipid analysis. Other laboratory tests performed were haemoglobin, white blood cell count erythrocyte sedimentation rate (ESR Westergren method), serum transaminases, alkaline phosphatases, serum creatinine, serum uric acid, fasting blood glucose, FBT and T3 resin test, and serum electrolytes. Urine was analysed for the presence of protein and glucose. All these tests were made by clinical routine methods in use at the hospital. At the same visit body weight was measured without overcoat and jacket. Half a kg was subtracted from the observed weight as an arbitrary weight for shoes and clothing. Body height was measured without shoes. All subjects were interviewed and examined by one of us (A.G.O.). A standardized clinical routine with a special medical record adjusted to be computerised was used.

Interview

Case history involved family history and questions were always asked concerning the known presence of HLP atherosclerotic manifestations, diabetes and thyroid diseases within the family.

Smoking habits, alcohol consumption and preceding illness were recorded. Direct questions were asked regarding the following diagnosis or symptoms: myocardial infarction, angina pectoris, congestive heart failure, cardiac arrhythmias, systemic hypertension, cerebrovascular disease, intermittent claudication, diabetes mellitus, liver disease or other major diseases such as pulmonary embolism and malignant disease. For chest pain on effort and intermittent claudication the Rose and Blackburn questionnaire (48) was essentially followed.

Questions were also asked as to current chronic drug treatment.

The physical examination

The physical examination included clinical routine examinations, a search for stigmata of HLP (corneal arcus, xanthelasma and tendon and skin xanthoma) and for signs of hypothyroidism.

Cardiovascular examination. The cardiovascular examination included auscultation of the heart, abdominal aorta, carotid, subclavian and femoral arteries. Blood pressure was measured in the right arm with a mercury manometer after five minutes rest in the supine position. The cuff was 13.5 cm wide. Readings were recorded at the nearest 5th mm Hg. The diastolic blood pressure was recorded at the disappearance of the 5th Korotkoff sound.

Exclusions

Patients with previous diagnoses or present symptoms of atherosclerotic diseases were excluded. The following diagnoses were found (numbers

within brackets) myocardial infarction (4) angina pectoris (3) congestive heart failure (1) cerebrovascular diseases (4) intermittent claudication (3) abdominal aortic aneurysm (1) and amaurosis fugax (1) Diagnosis was based on the history of the patient notes in the medical records of Metropoli and on hospital medical records

Subjects with disorders that could influence the interpretations of ECG recordings and peripheral circulatory investigation were also excluded. Diagnoses in this category were systemic hypertension (23) and cardiac arrhythmias (2) The definition of hypertension followed the criteria of Master et al (37).

In order to exclude secondary HL the following exclusions were made (number within brackets) history of or present diagnosis of dysfunction of the thyroid gland (18) diabetes (2) gout (4) liver (2) or renal (1) disease Patients with history of malignant disease (3) were all excluded. Patients treated with any of following drugs were excluded hypolipidaemic compounds (2) cardiac glycosides (5) diuretics (18) antihypertensives (3) adrenergic β receptor blocking agents (11) vasodilators (3) and hormones or agents with hormonal action including peroral contraceptives (13) Subjects were often excluded for more than one of the reasons mentioned. Subjects were not excluded cause of obesity

Other investigations

After the first visit to the Lipid Unit the subjects were referred for other investigations such as intravenous glucose tolerance test intravenous fat tolerance test exercise ECG chest x-ray and studies of the peripheral circulation. After these studies treatment of the HLP was begun.

The control groups

The control group was chosen to comprise subjects attending Metropoli having non-elevated serum cholesterol and TG concentrations. On at least two occasions they should have had fasting TG and cholesterol concentrations below 2.00 mmol/l or 300 mg/100 ml respectively Two hundred and twenty-two subjects with serum lipid concentrations below these limits were offered identical

investigations as the HLP subjects. Controls were age and sex matched to the HLP group. Exclusions were made according to the same criteria as in the HLP group. Twenty five subjects were excluded at the Lipid Unit because of diagnosis or drug treatment Encountered diagnoses or chronic drug treatments were cardiac valvular disease (1) cardiac arrhythmia (1) systemic hypertension (1) liver disease (1) kidney disease (6) thyroid disease (4) tuberculosis (4) malignant disease (3) diuretics (3) and estrogens (1) Of those offered examination 51 subjects (23 per cent) did not wish to participate 14 (6 per cent) did not reply and 4 (2 per cent) had moved away

RESULTS

The interspective sample

During the period of study 705 subjects exhibited "marked" HL of whom 314 HL subjects, 188 men and 126 women fulfilled the criteria and participated in the interspective study The formation of this "interspective sample" is detailed in Table II. After a certain time a reasonable number of males with type IV and females with type IIA had accumulated while other types were few At that stage because of capacity problems, subjects ($n=176$) with these frequent HLPs were not called to the interspective study Of the 420 subjects called to the interspective study 7 per cent did not wish to participate and 16 per cent were excluded at the Lipid Unit.

Table II Formation of the interspective sample. Number of subjects

Screened sample	20 132
HL	705
HL excluded at Metropoli because of diagnosis	109
HL not called to interspective study ¹	176
Total HL excluded from interspective study	285
HL called to interspective study	420
Not interested	30
Moved away	5
Dead	2
Excluded at the Lipid Unit	69
All excluded from Lipid Unit	106
INTERSPETIVE SAMPLE	314

¹ See text.

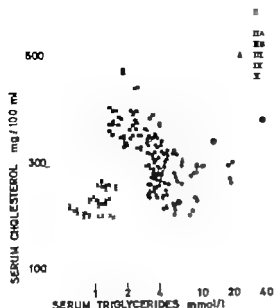


Fig. 3. Initial serum lipid concentrations in males in the interspective sample. C = control group, N = subjects with normal HLP type. II A, II B, III, IV and V indicate types of HLP (4).

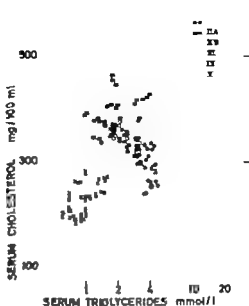


Fig. 4. Initial serum lipid concentrations in females in the interspective sample. See legend to Fig. 3.

The serum lipid values

Initial cholesterol and TG concentrations for men and women in HLP and control groups are given in Figures 3 and 4. High TG values were more abundant among males than females, while more females had high cholesterol values.

The difference between the first and second value for the serum lipids

The first and second serum cholesterol concentrations for the fasting subjects are given in Figure 5. The cholesterol values tended to be lower at the second determination. This tendency was more pronounced the higher the values. The number of males with higher/lower values for cholesterol at the second determination was in the cholesterol region below 300 mg/100 ml for the first determination 9/79 in the region 301–400 mg/100 ml 12/66 and for cholesterol above 400 mg/100 ml 1/13. The corresponding figure for females

were 9/7 21/53 and 1/11.

The plots for the first and second serum TG concentrations are given in Figure 5. There was a tendency also for lower serum TG concentrations at the second estimation that at the first and this tendency was greater the higher the initial serum TG. If the initial serum TG concentrations were divided into the regions below 2.00, 2.01 to 4.00 and above 4.00 mmol/l the number of males with higher/lower values for TG at the second determination was 19/6 19/45 and 15/45. The corresponding figures for females were 21/14 22/30 and 5/10.

DISCUSSION

The results of the procedure of serum cholesterol and TG screening of the Metropol sample show that it is possible to pick out subjects with primary HLP who have not experienced any symptoms of atherosclerotic disease.

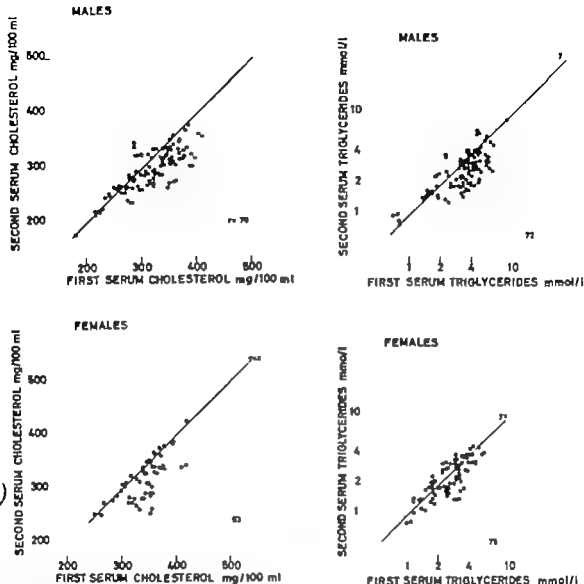


Fig. 5. Second (LP analysis) in relation to first (screening) fasting serum cholesterol and TG concentrations in males and females in the interpective sample.

This study did not aim at describing a "randomly selected" Stockholm population but to describe the conditions in a "health control sample". This sample is of great interest as it constitutes those subjects available for active preventive medicine.

The highly selective mechanism of the different stages involved to obtain this HLP group makes it impossible to draw any conclusions as to the prevalence of different types of HLP in Stockholm. The significant correlation between the first

and second serum cholesterol and TG concentrations suggests that most of these values reflect the habitual serum lipid concentrations of the subject. There was a fairly large variation between the first and second serum lipid value and a clear tendency towards lower values for both serum lipids at the second determination. Several mechanisms explain these facts. Both serum cholesterol and TG normally fluctuate and thus the phenomenon of "regression to the mean" will operate when sub-

jects are recruited by selecting those with the highest values. Also all subjects were informed at Metropol of their serum lipid concentrations and whether or not this concentration was elevated.

Some subjects therefore might have changed their dietary habits as the population in Stockholm in general is well informed concerning the relation between diet and HLP

FREQUENCY OF TYPES OF HYPERLIPOPROTEINAEMIAS AND SERUM LIPOPROTEIN CONCENTRATIONS, COMPOSITIONS AND INTERRELATIONS

LP analysis and typing of HLP was performed on sera of all HL subjects participating in the inter-spective study

METHODS FOR LIPOPROTEIN DETERMINATION

LP separations were made by preparative ultracentrifugation and paper electrophoresis. Serum was inspected after standing overnight at 4°C as a qualitative test for chylomicrons (4). The procedure of the preparative ultracentrifugation has been previously described in detail (9) and involves in essence the separation of the three LP classes normally present in the fasting state: very low density LP (VLDL) which corresponds to pre- β on electrophoresis, low density LP (LDL) corresponding to β -LP and high density LP (HDL) with α mobility on electrophoresis. After the first centrifugation at $d=1.006$ the top layer recovered from the centrifugation tube contains VLDL. The bottom fraction containing LDL and HDL is then centrifuged at $d=1.063$ and LDL and HDL are recovered in the top and bottom fraction of the tube respectively. To increase the density a solution of NaCl-NaBr is added. The three separated fractions VLDL, LDL and HDL are then extracted by isopropanol for determination of cholesterol (6) and TG (31) concentrations. In the LDL and HDL fractions bromide was removed from the isopropanol by the addition of 0.2 g Dowex 1-x8 (Cl) per ml. The results of the LP determination were accepted if regressions and correlations only if the recovery of cholesterol and TG in the three LP classes was 87–113 per cent of the total lipid concentrations. The average recovery in each of the six types of HLP varied between 93–99 per cent. For typing, lower or higher recoveries could

be accepted if the lacking or excess LP lipid could not affect typing.

LP paper electrophoresis was performed according to Lees and Hatch (34) on whole serum, and on top and bottom fractions after separation in the ultracentrifuge at $d=1.006$. Three bands: pre- β , β and α LP bands were visualized. Electrophoresis on the top and bottom fraction after separation at $d=1.006$ was performed in order to detect "floating- β " LP (see below).

TYPING OF HYPERLIPOPROTEINAEMIAS

Typing of the HLP serum was performed according to the principles of Fredrickson et al. (24) as modified in a WHO memorandum (4). Upper normal limits were based upon a normal adult male material in Stockholm where the LPs were determined by the same method as used here (15). Arbitrarily the upper limit was in rounded off figures set at the value for mean plus twice the standard deviation. The upper limits used were for LDL cholesterol 220 mg/100 ml and for VLDL TG 1.25 mmol/l. It is recognized that there are age and sex differences for the LP concentrations (17). We have deliberately only used the above values for all subjects, aiming at obtaining similar LP concentrations for the two sexes and all ages in the different types of HLP. The following criteria for classification into the different types of HLP were used.

Type I A HLP

1. LDL cholesterol concentration of 220 mg/100 ml or more.
2. VLDL TG concentration below 1.25 mmol/l.

Type II B HLP

1. LDL cholesterol concentration of 220 mg/100

- ml or more
2. VLDL TG concentration of 1.25 mmol/l or more
3. Presence of a pre- β LP and absence of a β -LP in the isolated VLDL on paper electrophoresis.

Type III HLP

1. Presence of "floating β " LP or " β -VLDL" visible as a band with β mobility on paper electrophoresis of the isolated VLDL.
2. VLDL TG concentration of 1.25 mmol/l or more.

Type IV HLP

1. VLDL TG concentration of 1.25 mmol/l or more.
2. LDL cholesterol concentration below 220 mg/100 ml.
3. Presence of a pre- β LP and absence of a β -LP in the isolated VLDL on paper electrophoresis.

Type V HLP

1. 2 and 3 as in type IV HLP
4. Fasting chylomicronaemia

"Normal" (N) type

1. VLDL TG concentration below 1.25 mmol/l.
2. LDL cholesterol concentration below 220 mg/100 ml.

The control group

In the control group, total serum cholesterol and TG estimation together with LP paper electro-

phoresis on whole serum was performed at the first visit. For practical reasons LP separation with preparative ultracentrifugation could not be made concomitantly with the other investigations of the controls. This was performed instead during the summer of 1974. Of the male and female controls, 78 and 88 per cent respectively returned for serum LP determination. The average time that had elapsed between the determination of total serum lipids and LP concentrations was 23 months. Mean body weight, mean total serum cholesterol and TG had not changed significantly in either sex during this period.

RESULTS

Frequencies of the different types of hyperlipoproteinaemia

In the interspective sample, the percentage frequencies of the different HLP types were (males/females) normal pattern (N) 19/25 type II A 16/37 II B 7/10 III 6/5 IV 50/24 and V 2/1. Type IV was thus by far the most common HLP type in males. N and type II A were of equal prevalence being about one-third as frequent as type IV. Type II B and type III were less common and type V was rare. In women the most frequent HLP type was II A while type IV was less abundant. The relative frequency of the other HLP types was of the same order as in men.

As stated above the choice of cut off points between "normal" and "elevated" levels is arbitrary. The effect of changing the cut off points for VLDL TG and LDL cholesterol on the allocation

Table III The effect of the use of different cut off points for defining elevated VLDL TG and LDL cholesterol on the distribution of the HLP subjects on different types. Number of subjects

	MEN			WOMEN		
VLDL TG mmol/l	1.10	1.25 ¹	1.40	1.10	1.25 ¹	1.40
LDL cholesterol mg/100 ml	200	220	240	200	220	240
N ²	22	35	49	III	31	49
II A ³	31	31	26	53	46	33
II B	40	III	3	27	12	8
IV	81	94	96	23	30	29
V	3	3	3	1	1	1

¹ Cut off points used for classification in this study

² N = normal LP type despite hyperlipidaemia at screening.

³ II A, II B, III, IV and V indicates type of hyperlipoproteinaemia (4).

of HLP subjects into different types of HLP is illustrated in Table III with one lower and one higher set of values. As expected, the higher the cut off point the more sera were classified as N. The most dramatic effect of going from low to high cut off points was in both sexes a pronounced reduction of the number of type II B. For type II A there was a sex difference so that the number of males was only reduced by 16 per cent going from the lowest to the highest cut off value while females were diminished by nearly 40 per cent. The number of subjects classified as having type IV HLP was slightly raised with increasing cut off points. The considerable differences in the number

of subjects allocated into different types of HLP especially type II B HLP when moderate changes were made in the cut off points demonstrate the arbitrary nature of the typing system.

Serum lipoprotein concentrations in the different types of hyperlipoproteinaemia

The individuals of each sex in the interspersive sample were allocated into 6 different groups according to type of HLP.

The distribution of the LP concentrations (Fig. 6-11) particularly LDL and VLDL, were strongly skewed which is partly a result of the fact

Table IV Mean, median and range of concentrations of cholesterol (chol, mg/100 ml) and triglycerides (TG mmol/l) in the different LP fractions of males with different types of HLP and in controls (C). N = normal type of HLP (in spite of HL at screening), n = number in each group. Chol/TG = mg/100 ml/mmol/l

Group	Estimate	Lipoproteins				LDL		HDL	
		VLDL							
		chol	TG	chol/TG		chol	TG	chol	TG
C n=46	Mean	12	44	27		125	32	62	15
	Median	12	37	—		125	31	58	15
	Range	4-30	10-127	16-60		79-189	17-53	38-97	.09-.27
N n=35	Mean	20	80	25		178	45	57	.21
	Median	20	90	—		183	46	53	.21
	Range	5-35	37-122	14-37		99-218	26-70	32-118	.09-.52
II A n=31	Mean	24	77	32		269	69	51	.21
	Median	23	82	—		260	70	51	.20
	Range	9-71	27-122	17-91		221-417	41-130	30-75	.06-.37
II B n=14	Mean	43	187	24		259	75	45	.25
	Median	43	162	—		257	74	45	.25
	Range	30-63	129-320	18-36		223-261	54-107	32-58	16-31
III n=11	Mean	99	295	33		339	64	42	.30
	Median	84	181	—		333	60	41	.26
	Range	47-287	154-1025	26-52		68-205	29-117	35-54	.22-.50
IV n=94	Mean	65	366	21		356	61	43	.30
	Median	54	268	—		362	58	43	.28
	Range	22-207	127-2153	8-38		63-219	29-109	25-69	.06-.79
V n=3	Mean	190	3675	12		62	64	34	.57
	Median	185	1015	—		60	72	35	.56
	Range	66-319	518-6492	5-18		49-76	39-81	22-45	.43-.72

See text, p. 20.

Table V Mean median and range of concentrations of cholesterol (chol, mg/100 ml) and triglycerides (TG mmol/l) in the different LP fractions of females with different types of HLP and in controls (C). See legend to Table IV

Group	Estimate	Lipoproteins				LDL		HDL	
		VLDL							
		chol	TG	chol/TG	chol	TG	chol	TG	
C n=61	Mean	13	46	28	131	.37	67	18	
	Median	12	.37	—	129	.35	64	16	
	Range	2-36	13-138	12-58	79-207	.20-.67	36-102	.07-.61	
N n=31	Mean	18	68	27	189	.53	66	23	
	Median	17	67	—	193	.52	63	23	
	Range	4-40	15-121	15-52	100-219	.23-.81	40-113	10-35	
II A n=46	Mean	22	67	37	262	.64	66	26	
	Median	22	66	—	249	.62	63	25	
	Range	7-40	18-119	20-58	222-412	.32-1.00	45-101	14-44	
II B n=12	Mean	44	1.75	25	253	.89	50	26	
	Median	42	1.68	—	261	.88	48	26	
	Range	33-72	1.31-2.68	20-35	220-288	.65-1.41	32-74	17-40	
III n=6	Mean	71	1.76	40	182	.83	45	26	
	Median	62	1.74	—	184	.89	44	26	
	Range	44-121	1.26-2.50	30-68	125-228	.50-1.08	38-58	18-36	
IV n=30	Mean	51	2.45	22	171	.74	50	33	
	Median	44	2.06	—	179	.70	48	29	
	Range	24-102	1.30-5.46	12-33	83-213	.37-1.53	32-85	17-85	
V n=1		128	15.17	8	81	.79	37	72	

† See text, p. 20.

that cut off points have been used for these LP concentrations in the formation of the groups. Therefore only mean, median, minimum and maximum concentrations for cholesterol and TG of the LPs in the different groups are given in Tables IV and V. In the control group which originally was defined as based upon total serum lipids, all subjects except one male (VLDL TG of 1.27 mmol/l) and on one female (VLDL TG of 1.38 mmol/l) had values for VLDL TG and LDL cholesterol which were below the upper normal limits used for these LPs.

VLDL concentrations. The distributions of the VLDL cholesterol and TG concentrations in the different groups are given in Figures 6 and 7 (Tables IV and V). By definition two groups — N and II A — had VLDL TG below 1.25 mmol/l. N

and II A were similar and differed from the control groups by having a greater frequency of higher values for VLDL TG and considerably higher mean/median values for both VLDL cholesterol and TG.

The three types having elevated VLDL TG had different distributions of this LP lipid. Type II B had the narrowest curve and the lowest mean/median value. Type IV had the broadest distribution while type III was intermediate.

The distribution curves for VLDL cholesterol in the different types paralleled in essence those of VLDL TG concentrations except for type III. Thus, there were relatively low VLDL cholesterol concentrations in controls, N and type II A HLP and higher values in type II B, III, IV and V HLP. In contrast to the distribution of VLDL TG the VLDL cholesterol showed a greater frequency of

MALES

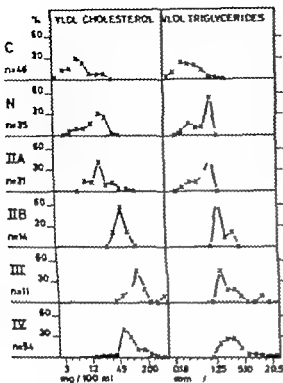


Fig. 6. Distributions of VLDL cholesterol and TG concentration in logarithmic scales in males in the different types of HLP: C = control group; N = normal LP type despite hyperlipidemia at screening (see text); II A, II B, III and IV = subtypes of HLP. The line indicates the cut off point used for defining VLDL TG in type IV of HLP.

high value in type III than in type IV. Also both mean and median values for VLDL cholesterol were greater in type III than in type IV while the opposite was true for VLDL TG. Therefore the highest ratio cholesterol/TG in VLDL was seen in type III HLP (Table IV and V). In both sexes also some subjects with HLP of type II A and N had relatively high concentrations of VLDL cholesterol in relation to the TG content resulting in a high cholesterol/TG also in VLDL.

Men tended to have higher VLDL TG and cholesterol concentrations than women in all HLP groups. This was most clearly seen in types III and IV.

LDL concentrations (Fig. 8 and 9, Table IV and V). By definition, types N and IV had LDL cholesterol below 220 mg/100 ml and types II A

and II B had values above this cut off point. For type III no criteria was applied with regard to LDL cholesterol, but only one – a female – of the 17 subjects with type III HLP had a LDL cholesterol above 200 mg/100 ml. As in the case of VLDL TG concentrations, there was a difference between controls and N with higher mean and median values for LDL cholesterol in the N groups. In both sexes the distribution of LDL cholesterol in type II A and type II B was different, type II A having broad and II B a steep and narrow distribution of LDL cholesterol.

In men low LDL cholesterol concentrations (below 100 mg/100 ml) (17A) were seen in 11 per cent of type IV subjects, 17 per cent of type III subjects and all 3 type V subjects compared to 27 per cent in controls. All these three types of HLP characterized by VLDL TG elevation had minimum values of LDL cholesterol below that of the

FEMALES

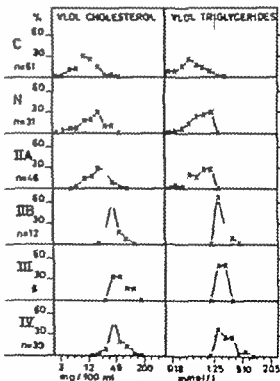


Fig. 7. Distributions of VLDL cholesterol and TG concentrations in logarithmic scales in females in the different types of HLP. See legend to Fig. 6.

MALES

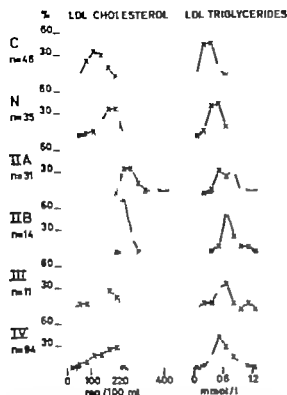


Fig. 8. Distributions of LDL cholesterol and TG in males in the different types of HLP. The line indicates the cut off point used for defining LDL cholesterol in typing of HLP. Symbols, see legend to Fig. 6.

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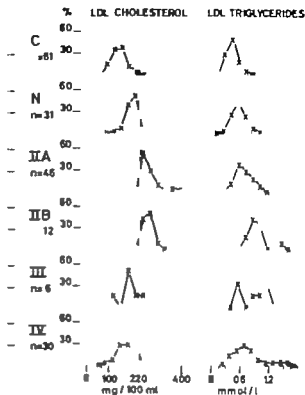


Fig. 9. Distributions of LDL cholesterol and TG in females in the different types of HLP. See legend to Figs. 6 and 8.

controls. The only woman with type V HLP had low LDL cholesterol concentrations, otherwise low values for this LP lipid were seen only occasionally in women. In control women it was found in 15 per cent.

The LDL TG concentration was higher than that of the controls in all types of HLP, thus also in types characterized by "normal" LDL cholesterol concentrations i.e. N, III, IV and V. Therefore, in contrast to VLDL, the situation of the distribution curves of LDL TG in the different types did not parallel those of LDL cholesterol. The highest mean LDL TG concentration was found in type IIB, where it was more than twice that of the control group.

LDL cholesterol and TG concentrations were slightly higher in women than in men in all types but type IIA.

HDL concentrations (Fig. 10 and 11). Types IV and V. Although HDL levels are not considered in the definition of the different types of HLP, several interesting differences were found between the different types. HDL cholesterol concentrations were lower in all HLP types than in the control groups. This was most pronounced for the types with an elevation of VLDL TG concentration. In these types mean HDL cholesterol was in women in no instance above 50 mg/100 ml while in the other groups it was above 65 mg/100 ml. In men the corresponding figures were 45 and 50 mg/100 ml. The highest individual values for HDL cholesterol concentrations were seen in the N types. Mean HDL cholesterol concentrations were higher in women than in men in all types.

In contrast to HDL cholesterol concentrations, HDL TG was higher in the HLP types with raised

MALES

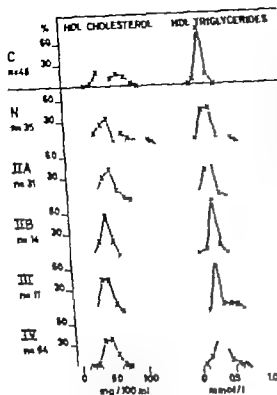


Fig 10. Distributions of HDL cholesterol and TG in males in the different types of HLP. Symbols, see legend to Fig. 6.

VLDL TG. The highest values for HDL TG were seen in type V. In both type IV and V values close to 1 mmol/l occurred in contrast to the mean value of 0.15 mmol/l in the control groups.

Lipoprotein lipid composition

The frequency distribution curves of cholesterol and TG in the LP classes indicated that in some types of HLP the relation between cholesterol and TG was different from that in other types. A LP class may consist of LP molecules of identical or different kinds. Within each kind of LP molecule the proportion of among other things cholesterol/TG is the same. If changes in the concentration of a LP class consisting of identical LP molecules were due to changes in the number of these molecules, the composition of the LP class such as e.g. the relation between cholesterol and TG

would therefore remain constant. The same would hold true if a LP class normally containing different kinds of LP molecules underwent a change in the number of molecules but with a retention of a constant ratio between the numbers of the different LP molecules. If however changes in the concentration were particularly due to increases/decreases in certain LP molecules with a different composition with regard to the ratio cholesterol/TG such concentration changes would be accompanied by alterations and disappearances in the constant relation between cholesterol and TG.

In order to analyze further the composition of the LP classes in the various types of HLP we considered all HLP as a one population, defined by the presence of HLP and performed correlation (Table VI) and regression analysis (Fig. 12-14) on the whole sample of HLP subjects for each LP class. The regression equation, if significant was

FEMALES

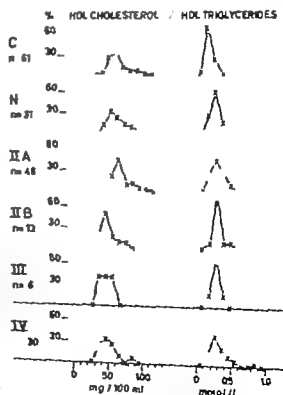


Fig 11. Distribution of HDL cholesterol and TG in females in the different types of HLP. Symbols, see legend to Fig. 6.

then regarded as a curve describing the average value for the "dependent" variable - cholesterol - in relation to the "independent" - TG. From the inspection of Figures 12-14 one could then evaluate whether all types of HLP had the same relation between cholesterol and TG in a LP class, I = were distributed equally on both sides of the least squares curve.

VLDL composition. For VLDL (Fig. 12 Table VI and VII) there was a close positive correlation between its content of cholesterol and TG. For practical purposes, the relation is given with logarithmic values. Both linear and quadratic regres-

sions were employed and the quadratic term was significant ($p < 0.01$) for both males and females. When non-logarithmic values were used the regression equation contained the origin. There was no difference in the relationship between male and female VLDL. All types of HLP except type III fitted into the same relation between cholesterol and TG. For type III the cholesterol content was consistently higher in relation to the TG content than the average relation would have predicted. However a few type II A and some types IV HLP had a similar high cholesterol content in relation to the TG content in VLDL. Exclusion of type III

Table VI. Correlation coefficients between LP lipids

	\log VLDL TG	LDL cholesterol	LDL TG	HDL cholesterol	HDL TG
MALES n 135					
\log VLDL cholesterol	90	-.75	.09	-.47	.55**
\log VLDL TG		-.77	.01	-.46	.58**
LDL cholesterol			.21	.42***	-.54
LDL TG				-.10	.22**
HDL cholesterol					-.093
FEMALES n 84					
\log VLDL cholesterol	93	-.59	.52**	-.60**	.45**
\log VLDL TG		-.65**	.40*	-.59	.49**
LDL cholesterol			-.044	.37	-.26
LDL TG				-.26	.38
HDL cholesterol					.011

and *** significant correlations on the 5, 1 and 0.1 percent level respectively

Table VII. Equations for the dependence of different LPs on \log VLDL TG in mmol/l (x) in subjects with HLP

MALES			FEMALES		
#	Equation	P	Equation	#	2
\log VLDL cholesterol mg/100 ml	$y = 3.343 - 0.00416 x^2$	< 0.001	$y = 3.365 - 0.083 x^2$	< 0.001	< 0.005
LDL cholesterol mg/100 ml	$y = 231 - 62.0 x$	< 0.001	$y = 233 - 48.9 x$	< 0.001	-
LDL TG mmol/l		> 0.05	$y = 0.71 + 0.126 x$	< 0.001	-
HDL cholesterol mg/100 ml	$y = 49 - 5.4 x$	< 0.001	$y = 58 - 11.7 x$	< 0.001	-
HDL TG mmol/l	$y = 0.22 + 0.020 x^2$	< 0.005	$y = 0.26 + 0.037 x^2$	< 0.001	< 0

Both linear and quadratic regression was applied. The former is given when the quadratic term was not significant.

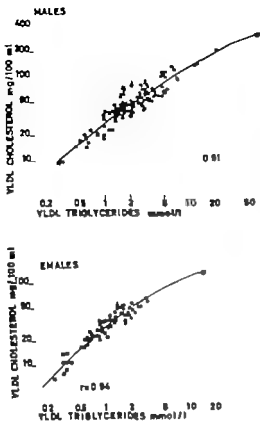


Fig. 12. VLDL cholesterol in relation to VLDL TG in males and females with HLP. VLDL cholesterol and TG are given in log scales. The lines are the regression lines of log VLDL cholesterol on log VLDL TG. Symbols as in fig. 3.

from the correlation analysis did not, however, effect r values of the regression equations probably due to the low number of type III HLP.

Thus, it appears that in types II A, II B, IV and V we have VLDL LP with the same relation between cholesterol and TG suggesting that the level of VLDL is directly determined by the number of similar VLDL particles. It has to be considered that the relation was not linear but rather of a parabolic nature so that at higher VLDL TG there was proportionally less cholesterol. This might be explained if the VLDL particles increased in size with rising VLDL concentrations as has been reported (49). This would imply decrease of surface (rich in free cholesterol and protein) to volume (TG rich). In type III HLP the VLDL particles are more cholesterol rich, which

has previously been described (29).

LDL composition. For LDL the relation of cholesterol to TG was positive but weak for males and insignificant for females. Figure 13 shows that there were several differences between the types of HLP in both males and females for this relation. Type V had a very low LDL cholesterol in relation to its LDL TG concentration. Type III had also a consistently low cholesterol concentration in relation to its TG content but type V was further below the line than type III. Type II B and even more type II A had high LDL cholesterol in relation to LDL TG. Some type II A who clinically had tendon xanthomata which have been called type II A-X (14) had a particularly high cholesterol content in relation to the TG content.

Figure 13 suggests that there was a strong positive linear relationship between the cholesterol

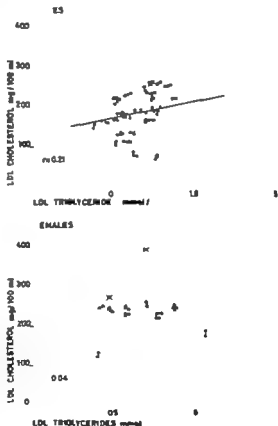


Fig. 13. LDL cholesterol in relation to LDL TG in males and females with HLP. The line is the regression line of LDL cholesterol on LDL TG (only significant in males). Symbols as in fig. 3. X indicates II A-X (see text).

and TG concentrations of LDL for type III HLP. The existence of such a relation was studied by linear regression analysis separately for the various types. There were no major differences between males and females. The r -values for the relation between LDL cholesterol and TG were (both sexes pooled) for types II A, II A-X, II B III and IV 0.17 0.52, 0.37 0.83 ($p < 0.001$) and 0.02 respectively.

The weak or non-existing relationship between cholesterol and TG in LDL shows that this LP class was not composed of one species of well defined LP molecules in the entire HLP population. There was a clear tendency however for all types except type IV to show a fairly characteristic LDL composition with either high or low cholesterol content in relation to the TG content. Our LDL LP is defined as having a density range of 1.006–1.063. Within this range there are at least two different LP classes of different composition, LDL₁ ($d = 1.006$ – 1.019 S_f 12–20) and LDL₂ ($d = 1.019$ – 1.063 S_f 0–12) the former being more TG rich, the latter more cholesterol rich. It is thus possible that the two extremes with regard to LDL composition, type V and type II A X had mainly LDL₁ respectively LDL₂ in the isolated LDL. Gofman and co-workers in their early work with the analytical ultracentrifuge showed that patients with tendinous xanthomata indeed had a great crease of the S_f 0–12 LP (26).

Type III HLP the diagnosis of which was based on two VLDL criteria only also showed a characteristic LDL composition with LDL rich in TG in relation to its cholesterol content. Furthermore LDL from type III HLP was the only LDL fraction having a significant correlation between its TG and cholesterol content ($r = 0.83$) suggesting that type III LDL is the most homogeneous LDL LP at least with regard to the cholesterol/TG relation.

HDL composition. For HDL (Fig. 14) there were no significant correlation between its cholesterol and TG content for the whole HLP population. Only for type III was there a correlation $r = 0.81$ for men. Type V was characterized by a low cholesterol and high TG content. Females with type II A had a higher cholesterol content than the types with VLDL elevation in relation to the TG concentration. This was not so apparent for males.

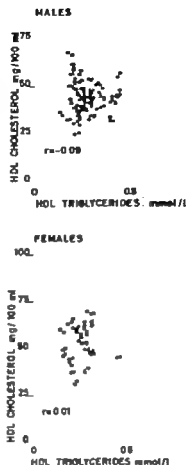


Fig. 14. HDL cholesterol in relation to HDL TG in males and females with HLP. Symbols as in Fig. 3.

Some caution must be exercised in the interpretation of these data as the concentration of HDL TG is very low and thus subject to analytical errors. Furthermore there may be some uncertainty with regard to the specificity of the TG method in this fraction as the HDL LP was not isolated from the other serum constituents. However the lack of correlation between HDL cholesterol and TG suggests heterogeneity of this LP except for type III HLP.

Continuous relation for lipoprotein classes between types of hyperlipoproteinaemia

VLDL and LDL are the two classes of LP used in the classification of HLP. Since TG is the major component of VLDL and cholesterol that of LDL,

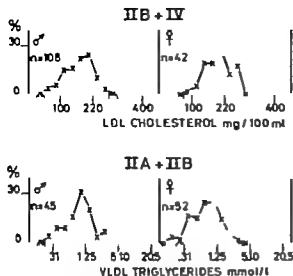


Fig. 15. Distribution of LDL cholesterol in males and females with elevated VLDL TG concentration (above) and of VLDL TG in males and females with elevation of LDL cholesterol levels (below).

VLDL TG and LDL cholesterol have been used for the classification. When VLDL is "elevated" this HLP is further subdivided into type IV with normal LDL and type II B with raised LDL. However the distribution curves for LDL cholesterol were skewed to the left and to the right respectively for these two types, and in fact when they were pooled the common LDL cholesterol distribution curve (Fig. 15) for HLP with elevation of normal (pre- β moving) VLDL did not indicate the presence of two LDL populations. On the contrary the LDL distribution curve was continuous and fairly normal. A similar picture evolves if we consider instead the HLPs with "raised" LDL cholesterol. They are also subclassified into two types, II A with "normal" VLDL and II B with raised VLDL. If we look at the distribution curves of VLDL TG in these two types of HLP they were skewed to the left and right respectively. When they were pooled the common distribution curve for VLDL TG for all HLPs with raised LDL cholesterol (Fig. 15) did not show any significant signs of bimodality but was fairly continuous.

From these fairly continuous distribution curves the cut off points used for both LDL and VLDL seem arbitrary and perhaps even artificial. Further

more, it was clear from the description of the different types of HLP that some LP characteristics, for example low HDL cholesterol were related more to a certain LP abnormality (VLDL TG elevation) than to a specific type of HLP.

These characteristics suggest the existence of continuous relations between the various LP classes in this material of HLP. Since the classification of HLP into rigid types breaks any such relations, as we have discussed previously (70) we have analyzed the presence of such continuous relations between VLDL and the other LP classes in all pooled HLP subjects of each sex.

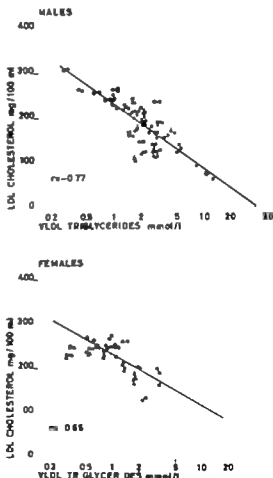


Fig. 16. LDL cholesterol in relation to VLDL TG in males and females with HLP. VLDL TG are given on log scale. The lines are the regression lines of LDL cholesterol on log VLDL TG. Symbols, see Fig. 3.

Relation between different lipoproteins

Correlation coefficients between different LP lipids are given in Table VI. VLDL was fairly strongly correlated to both LDL and HDL. These relations are therefore further illustrated in Figures 16–19. Equations are given in Table VII. LDL cholesterol was negatively related to VLDL TG concentrations (Fig. 16) and the regression equations were almost identical in both sexes. Except at the highest levels of VLDL TG, LDL cholesterol could vary within rather wide limits. However at every level of VLDL TG the maximal LDL cholesterol concentration seemed to be very definitely determined. Therefore only low and never high LDL cholesterol concentrations were seen with high VLDL TG levels. All types of HLP appeared to fit into this regression except II B which was above the line in both sexes. This is explained by the fact that this type by definition has an elevation of both VLDL TG and LDL cholesterol.

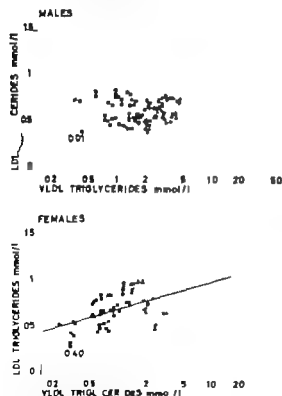


Fig. 17. LDL TG in relation to VLDL TG in males and females with HLP. VLDL TG are given in log scale. The line is the regression line of LDL TG on log VLDL TG (only significant in females). Symbols, see Fig. 3.

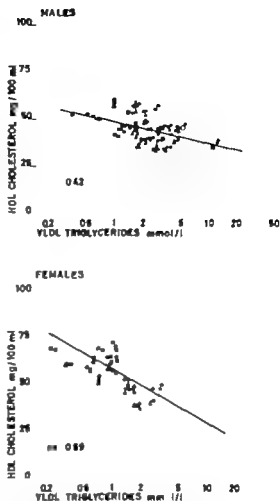


Fig. 18. HDL cholesterol in relation to VLDL TG in males and females with HLP. VLDL TG are given in log scale. The lines are the regression lines of HDL cholesterol on log VLDL TG. Symbols, see Fig. 3.

The relation between VLDL and LDL TG (Fig. 17) was positive but weak in females, and not significant in males. There did not appear to be any type specific relation.

Negative correlations existed in both sexes between VLDL TG and HDL cholesterol (Fig. 18). As a result of this, HDL cholesterol was decreased in types of HLP with VLDL TG elevation (II B, III, IV and V). All types of HLP fitted into the general relationship.

On the other hand, positive correlations existed between VLDL and HDL TG (Fig. 19). This was best described by a parabolic function with a positive quadratic term when VLDL TG was loga-

rithmic and HDL TC linear. Once again the relation fitted for all types of HLP.

Classification of hyperlipoproteinaemia based on serum lipid concentrations

For practical purposes it is of interest to know to what extent the determination of only serum lipids can lead to the correct diagnosis of type of HLP. Thus it has been suggested to divide HL into three groups according to the concentrations of cholesterol and TG: 1) elevation of only cholesterol, 2) elevation of only TG, and 3) elevation of both lipids (10, 19). Groups 1 and 2 have then been believed to represent type II A and IV respectively, and group 3 type II B and other types such as III and IV.

The value of total serum lipids in the diagnosis of types of HLP has been evaluated here by relating the values for total serum TG and cho-

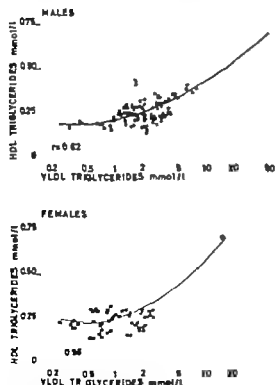


Fig. 19. HDL TG : relation to VLDL TG in males and females with HLP. VLDL TG are given in log scale. The lines are the regression lines of HDL TG on log VLDL TG. Symbols, see Fig. 20.

MALES

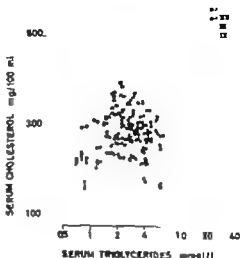


Fig. 20. Serum cholesterol and TG concentrations in men with HLP and controls at the LP determination.

FEMALES

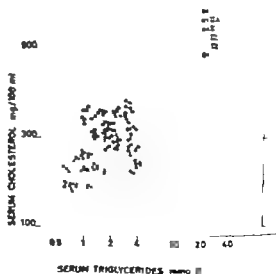


Fig. 21. Serum cholesterol and TG concentrations in women with HLP and controls at the LP determination.

lesterol to the actual type of HLP (Fig. 20 and 21). If we choose the upper limits of 320 mg/100ml for cholesterol and 2 mmol/l for TG it can be seen that lipid group 1) comprised only type II A. Group 2) comprised mainly type IV but also types II A, II B, III and V. Group 3 also comprised all types of HLP. This clearly shows how incomplete the picture is when only serum TG and cholesterol are determined. The importance and necessity of LP analysis for correct diagnosis is clearly evident.

DISCUSSION

Classification of hyperlipoproteinaemia

Five different types of HLP (type II A, II B, III, IV and V) could be identified in our sample of HL subjects. In addition 20–25 per cent of the subjects with HL at screening had VLDL TG and LDL cholesterol concentration within normal limits at the second study and were thus classified as having a normal (N) LP pattern. This finding has probably several explanations. Both total serum cholesterol and TG concentrations tended to be lower at the second blood sampling when blood was drawn for serum LP analysis for reasons discussed above. It is likely that the decrease in total serum cholesterol and TG concentrations effects decreases in LDL cholesterol and TG as well as in VLDL TG and cholesterol levels. Thus, in a number of subjects these concentrations could have decreased beneath the cut off points for HLP. However in addition as seen in Figures 10 and 11 the subjects with the highest HDL cholesterol concentrations were found in the N groups. As HDL cholesterol is part of total serum cholesterol it is possible that some subjects already at the first blood sampling had hypercholesterolaemia due to hyper HDL (α)-cholesterolaemia with LDL cholesterol concentrations within normal limits. Also in the typing of HLP small changes in the cut off points for VLDL TG and LDL cholesterol greatly affect the allocation of subjects with hyperlipidaemia into different types of HLP. Thus, as illustrated in Table III changing the cut off points for VLDL TG and LDL cholesterol from 1.10 mmol/l and 200 mg/100 ml to 1.40 mmol/l and 240

mg/100 ml doubled to tripled the number of subjects with "normal" LP pattern.

Advantages and disadvantages of the present system for classification of hyperlipoproteinaemia

The present typing system for HLP was suggested by Fredrickson, Lees and Levy (24) and has been slightly modified (4). It has several advantages. One major advantage is that it stimulates thinking in terms of LP instead of total lipid concentrations. Another is its simplicity in the sense that it facilitates communication. Indeed it is easier to say and write type II B instead of "hyper VLDL-lipoproteinaemia combined with hyper LDL-lipoproteinaemia".

However the typing system also has several drawbacks. It has been argued that individuals often oscillate between several types of HLP within a short time. Also, one may easily become inclined to look erroneously at the types of HLP as separate disease entities. An example of this attitude is the expression "type II B disease". However it has to be borne in mind that the typing system has nothing to do with etiology and pathogenesis and that the typing is done on the plasma sample and not on the patient (four P problem, 13).

In the present study some other disadvantages of the typing system were evident.

1. By necessity the cut off points must be arbitrarily defined as the distribution curves of the LPs are unimodal and continuous.
2. The allocation of subjects into different types of HLP was highly dependent on the cut off points chosen, not only in the sense that increasing or decreasing the cut off points altered the number of subjects having HLP but also in that the relative frequencies of the various types changed. (Table III)
3. From Figure 15 it was evident that looking at all subjects with elevation of LDL cholesterol or VLDL TG an approximately "normal" distribution is achieved with the other diagnostic LP. For example VLDL TG in type II A and II B formed together a "normal" continuous relation.
4. A continuous relation existed between LDL cholesterol and VLDL TG in the HLP popula-

tion (Fig. 16). This smooth relation is indeed completely interrupted by the use of cut off points for these LP lipids and misleadingly transforms the continuous relation into discrete populations.

These findings make the typing system appear highly arbitrary even artificial and sometimes misleading.

Metabolic aspects on lipoprotein composition and lipoprotein interrelations in the different types of hyperlipoproteinemia

The metabolic functions of serum LP is to transport fats between tissues. One major route in the fasting state is transport of TG synthesized from fat or carbohydrates, from the liver to peripheral tissues for oxidation and storage (47). The LP metabolism during this transport is schematically depicted in Figure 22. In essence the TG are synthesized in the liver and incorporated into VLDL, which are secreted from the liver into blood. In blood they pick up apolipoproteins from HDL (5). The TG rich VLDL is then attacked by the enzyme system lipoprotein lipase which splits off fatty acids from the TG. By this process VLDL loses more and more TG and eventually becomes degraded to LDL_1 . LDL_1 the final stage of this TG transport is the catabolized. The details of these processes are not known. It is believed that the first step when VLDL is attacked by lipoprotein lipase and loses TG leads to the formation of "intermediate particles" (45) which normally are metabolized rapidly to LDL by for instance lipase activities. During these processes apolipoprotein leaves the less and less TG rich LP and is transferred to HDL (23).

We want to briefly discuss our major findings of LP concentrations, LP composition and LP interrelations against this schematic metabolic background assuming first order kinetics to exist at all levels.

Type II A

Type II A defined as raised LDL cholesterol, had a LDL characterized by high cholesterol content in relation to TG content. This is probably due to accumulation of LDL_2 the final product in the

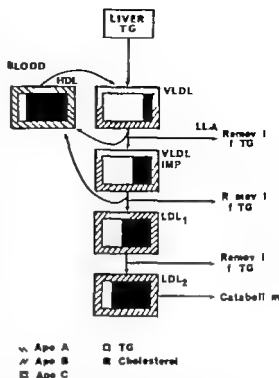


Fig. 22. Schematic picture of the metabolic interrelation of VLDL, LDL and HDL in the transport of TG from the liver. VLDL - IMP VLDL-intermediate particles, LLA lipoprotein lipase activity Apo A, B, C apolipoproteins A, B, C.

long chain of events in VLDL/LDL metabolism. The most obvious kinetic explanation for rise in only this LP class would be a specific block in the catabolism of LDL_2 . In fact studies with LDL labelled in the protein moiety with ^{35}S have shown that the fractional LDL catabolism is reduced in type II A-X patients compared to normal subjects (32). In accordance with this concept of a simple defect in catabolism of LDL_2 there were no general abnormalities in HDL or VLDL in the groups with type II A. However some subjects tend to have increased cholesterol/TG ratio in VLDL. This might suggest a defect in the handling of VLDL-intermediate particles (see below under type III).

Type II B

Type II B defined by raised LDL cholesterol and VLDL TG had compared to type II A a LDL

characterized by a higher TG content in relation to its cholesterol content. In fact II B had the highest LDL TG. This suggests that type II B had an accumulation not only of LDL₂ but also of the TG rich LDL₁. A combination of raised VLDL, LDL₁ and LDL₂ is from the kinetic point of view most easily explained by an increased production by the liver of VLDL which would increase the turnover and concentration of these LP classes. The changes seen in HDL in this type are characteristic for the elevation of VLDL and will be discussed when the relation between VLDL and HDL is discussed.

Type III

Type III defined by raised VLDL TG and presence of a β -VLDL¹ which is believed to represent the VLDL-intermediary particle (45) had a TG rich LDL which over its entire range of concentration has a strong correlation between its cholesterol/TG content. This LDL composition may fit with the accumulation of a TG rich LDL₁ of fairly constant composition which may be closely related to the VLDL intermediary particles. In fact Gofman et al demonstrated that patients with xanthoma tuberosum who thus probably had type III HLP had raised S_f 12-20 (LDL₁) and 20- (VLDL) LP (26). The rise in not only VLDL TG also in the ratio cholesterol/TG in VLDL₁ suggests the accumulation of VLDL-intermediary particles. The metabolic block in type III would then lie in the conversions of VLDL-intermediary and LDL leading to accumulation of these LPs.

Type IV

Type IV defined by elevation of VLDL TG showed a wide range of LDL cholesterol and TG concentrations and also a wide range in the relation between these two LDL lipids. This suggests a great variation in the relation between LDL₁ and LDL₂ in this HLP. A simple explanation for the rise in VLDL TG in type IV would be a block in the catabolism of VLDL. All other things being unchanged this would not affect the others LPs. In fact several type IV HLP had fairly normal concentrations and composition of LDL. However

many also had low to very low LDL cholesterol and high LDL TG in relation to the cholesterol content thus suggesting low LDL₂. The negative relation between VLDL TG and LDL cholesterol suggests that LDL₂ decreased particularly at high VLDL TG. There are two main possible reasons for a low LDL₂. 1) Increased fractional catabolism of LDL₂. 2) Decreased formation of LDL₂. At least two mechanisms seem possible for a decreased formation. The first is a decreased production (turnover) of VLDL TG e.g. by feed-back inhibition of secretion from the liver by the high VLDL levels. The second is a decreased formation of LDL₂ at a normal VLDL TG turnover. This may occur if the VLDL particles were larger than normal thus having a decreased surface/volume ratio. As the hydrophobic TG probably are in the interior and the hydrophilic β protein and the free cholesterol on the surface large VLDL would imply lower content of β protein the protein precursor of LDL₂ per unit of TG mass. Thus for the transport (turnover) of the same amount of TG less β protein and thus less LDL₂ would be formed.

It is also possible that in some type IV where both LDL cholesterol and TG were high - although not elevated - the kinetic mechanism for the HLP was an increased formation of VLDL as is suggested for type II B.

The pathogenesis of type IV HLP has not been elucidated. Some studies indicate that the major metabolic defect is reduced catabolism of VLDL (8-28) while others favour an increase in VLDL production (1-46). It is quite possible that these discrepancies in part may be due to a considerable heterogeneity of type IV HLP with various pathogenic mechanisms behind the increase in VLDL TG. It is possible that consideration of LDL concentration and composition as discussed above may help to distinguish between type IV HLP due to either defect catabolism of or to increased synthesis of VLDL.

Type V

Type V defined by the presence of chylomicrons in fasting serum and raised VLDL, had a LDL characterized by very low cholesterol concentration and a high TG content in relation to the

cholesterol content, suggesting very low LDL₂ levels. It is believed that chylomicrons and VLDL are at least initially catabolized by the same mechanisms involving lipoprotein lipase activity (47). The most simple mechanism for this HLP from the kinetic point of view would be a block in the first step of VLDL and chylomicron degradation. It is possible that the catabolic block in VLDL and chylomicron metabolism is more severe than in type IV. The probably very low LDL₂ levels may have the same two explanations as discussed above for type IV. Both in type IV and type V turnover studies are needed to resolve these problems.

The postulated decrease in catabolism of VLDL and chylomicrons may both in type IV and V be due to a decrease in lipoprotein lipase activities (7, 44) or to reduced fatty acid incorporation into adipose tissue (FIAT) function (21).

Lipoprotein relationships in hyperlipoproteinemia

Both LDL and HDL showed strong interrelations to VLDL. In LDL this was particularly true for LDL cholesterol showing a negative correlation while LDL TG had no or a weak, positive correlation. This suggests from the compositional aspects discussed above that LDL₂ was strongly negatively correlated to VLDL. We know that at the beginning of this relation (Fig. 16) with high LDL cholesterol and normal VLDL TG (II A) the fractional catabolism of LDL is decreased (3). One simple explanation for the decline in LDL cholesterol with rising VLDL would be that when VLDL TG increases there is first a normalization and then an increase in the fractional catabolism of LDL. In fact when moving along this line from type II A, one first encounters type IIB and types we assumed a normal LDL catabolism (and raised VLDL turnover) in the discussion above. Further on along the line we encounter the severe type IV and type V (which types we have already discussed the possibility of an increased fractional catabolism of LDL). To summarize the simplest explanation for the negative correlation between VLDL TG and LDL cholesterol would be a continuous increase in LDL fractional catabolism from reduced via normal to increased values with rising

VLDL TG. This hypothesis needs turnover studies to be validated. Needless to say there are other but more complex possible mechanisms.

VLDL had different relations to the two HDL lipids. There existed a positive relation to HDL TG. A similar relation was found by Lindgren et al. (36) in 16 healthy subjects. The role of HDL in TG transport is far from clear. However HDL TG increases during alimentary lipaemia (38). Further more, during in vitro incubation of human serum, TG is transferred from VLDL to HDL in exchange for cholesterol esters (39).

HDL cholesterol, on the other hand, had a negative correlation to VLDL TG. A negative correlation between serum TG and HDL cholesterol has been reported previously (11). The cause of this negative relation is not known. Some of the HDL may be combined to VLDL (25) (Fig. 22) and thus at high VLDL levels the HDL concentration may decrease. It is also possible that low HDL cholesterol may be a primary factor in the HLPs with VLDL elevation. At least one component of the HDL system, apolipoprotein C is of importance for VLDL catabolism as it is an activator of the lipoprotein lipase activity (33). Furthermore HDL is important in the LCAT reaction (41) which possibly also is involved in the catabolism of VLDL (41, 51).

SUMMARY

In order to study lipoprotein (LP) abnormalities in asymptomatic subjects with hyperlipidaemia, serum cholesterol and triglycerides (TG) were determined in about 70 000 active professional men and women attending health control linked to their employment.

Quantitative LP analysis with preparative ultracentrifugation, separating very low (VLDL), low (LDL) and high (HDL) density LP as subsequently performed on the sera of 314 asymptomatic subjects, who at the screening had serum cholesterol ≥ 350 mg/100 ml and/or TG ≥ 1 mmol/l. Cholesterol and TG concentrations were determined in each of the LP classes. Paper electrophoresis was run on whole serum and on the ultracentrifuge at $d=1.006$. Type

REFERENCES

- 1 Adams, P. W. Kimbark, A. H. Harrison, P. Stokes, T. and Wynne, V. The kinetics of plasma free fatty acid and triglyceride transport in patients with idiopathic hypertriglyceridaemia and their relation to carbohydrate metabolism. *Europ. J. Clin. Invest.* 4:149 1974
- 2 Adielsberg, D. and Sobotka, H. Pathological manifestations of abnormal cholesterol metabolism. I. Cholesterol, p. 375 (Cook, R. P. Ed.) Academic press, New York and London, 1958.
- 3 Albrink, M. J. and Man, E. B. Serum triglycerides in coronary artery disease. *Arch. int. Med.* 103:4 1959
- 4 Beaumont, J. L. Carlson, L. A. Cooper, G. R., Fajfar, Z., Fredrickson, D. S. and Strasser, T. Classification of hyperlipidaemias and hyperlipoproteinaemias. *Bull. Wild Hlth Org.* 43:891 1970.
- 5 Bilheimer, D. W. Eisenberg, S. and Levy, R. L. The metabolism of very low density lipoprotein proteins. I. Preliminary *in vitro* and *in vivo* observations. *Biochim. Biophys. Acta* 260:212, 1972.
- 6 Block, W. D. Jarrett, R. J. and Leoline, B. Use of a small color reagent to improve the automated determination of serum total cholesterol. I. A toluene in analytical chemistry vol. 1 p. 345 (Skoggs, L. T. Ed.) Mediad Inc. New York, 1965.
- 7 Carlson, L. A. Hepatin-released blood plasma lipoproteinase activity in patients with hyperlipoproteinaemia. *Acta med. scand.* 191:97 1972.
- 8 Broberg, J. Carlson, L. A., Pryor, U. Larsson, B. W. Wahlqvist, M. L. Splanchnic secretion rates of plasma triglycerides and total and splanchnic turnover of plasma free fatty acids: men with normo- and hypertriglyceridaemia. *Europ. J. Clin. Invest.* 2:454 1972
- 9 Carlson, L. A. Lipoprotein fractionation. *J. Int. Path.* 26, suppl. (Ass. Clin. Path.) 5:32, 1973.
- 10 Carlson, L. A. Serum lipid in normal men. *Acta med. scand.* 167:366, 1960.
- 11 Carlson, L. A. Serum lipid in men with myocardial infarction. *Acta med. scand.* 167:399 1960.
- 12 Carlson, L. A. Determination of serum triglycerides. *J. Atheroscler. Res.* 3:334 1963.
- 13 Carlson, L. A. Plasma or patient paper-electrophoresis: physica? The four-P problem in identification of hyperlipidaemia. *Atherosclerosis*, Editorial, 12:81 1970.
- 14 Carlson, L. A. Serum lipoprotein composition in different types of hyperlipoproteinaemia. I. Proceedings of the V International Symposium on Drugs Affecting Lipid Metabolism. (Holmes, W. Kritchevsky D. and Paoletti, R. Eds) In press.
- 15 Carlson, L. A. Unpublished data.
- 16 Carlson, L. A. and Böttig, L. I. Ischaemic heart disease in relation to fasting values of plasma triglycerides and cholesterol. The Stockholm Prospective Study. *Lancet* 1:863 1972.
- 17 A. Carlson, L. A. and Ericsson, M. Quantitative and qualitative serum lipoprotein analysis. I. Studies in healthy men and women. *Atherosclerosis*. 1975 In press.
- 17 B. Carlson, L. A. and Ericsson, M. Quantitative and qualitative serum lipoprotein analysis. II. Studies in male survivors of myocardial infarction. *Atherosclerosis*. 1975. In press.
- 18 Carlson, L. A. and Lindstedt, S. The Stockholm Prospective Study. I. The initial values for plasma lipids. *Acta med. scand. Suppl.* 493 1968.
- 19 Carlson, L. A. and Olsson, A. G. Hypertriglyceridaemia and its management. In: *Modern trends in cardiology - 3* p. 405 (Oliver, M. F. ed.) Butterworths, London, 1975
- 20 Carlson, L. A., Olsson, A. G., Oro, L., Rösner, S. and Walldius, G. Effects of hypolipemic regimes on serum lipoproteins. In: *Atherosclerosis III. Proceedings of the third international symposium*, p. 768. (Schettler, G. and Weizel, A., Eds.) Berlin, Heidelberg, New York, Springer Verlag, 1974
- 21 Carlson, L. A., Walldius, G. and Olsson, A. G. Evidence for defect in fatty acid uptake by adipose tissue of patients with hypertriglyceridaemia. *J. Clin. Path.* 26, suppl. (Ass. Clin. Path.) 5:88, 1973.
- 22 Davoon, W. J. and Massary, J. J. Jr. Introduction to statistical analysis, p. 249. International Student Edition. McGraw-Hill, Kogakusha.
- 23 Eisenberg, S., Bilheimer, D. W. and Levy, R. L. The metabolism of very low density lipoprotein proteins. II. Studies on the transfer of apoproteins between plasma lipoproteins. *Biochim. Biophys. Acta* 280:94 1972.
- 24 Fredrickson, D. S., Levy, R. L. and Lees, R. S. Fat transport in lipoproteins: an integrated approach to mechanisms and disorders. *New Engl. J. Med.* 276:34 94 148, 215 273 1967
- 25 Imms, R. H., Hoard, R. P., Lakshmi, K. and Norcia, L. N. The serum lipid and lipoprotein in normal and hyperlipidaemic subjects as determined by preparative ultracentrifugation. *Am. J. Clin. Nutr.* 9:79 1961
- 26 Gofman, J. W. et al. The serum lipoprotein transport system in health, metabolic disorders, atherosclerosis and coronary heart disease. *Plasma* 4:113, 1954.
- 27 Goldstein, J. L., Hazzard, W. R., Schrott, H. G., Bierman, E. L. and Motulsky, A. G. Hyperlipidaemia in coronary heart disease. *J. Clin. Invest.* 52 1533 1973.
- 28 Havel, R. J., Kane, J. P., Balazs, I. O., Segel, N. and Basco, L. V. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridaemic and hypertriglyceridaemic man. *J. Clin. Invest.* 49:2017 1970.

29. Hazzard, R. H., Port, D. J. and Bierman, E. L. Abnormal lipid composition of very low density lipoproteins in diagnosis of broad-beta disease (Type III hyperlipoproteinemia). *Metabolism* 21:1009 1972.
30. Kannel, W. B., Dawber, T. R., Friedman, G. D., Gleason, W. E. and McNamara, P. M. Risk factors in coronary heart disease. An evaluation of several serum lipids as predictors of coronary heart disease. *Ann. Int. Med.* 61:338, 1964.
31. Kessler, G. and Lederer, H. Fluorometric measurements of triglycerides. In: *Automation in analytical chemistry* vol. 1, p. 341 (Slagter, L. T. Ed.). Mediad Inc., New York, 1965.
32. Langer, T., Strober, W. and Levy, R. I. The metabolism of low density lipoprotein in familial type II hyperlipoproteinemia. *J. Clin. Invest.* 51:1328, 1972.
33. La Rose, J. C., Levy, R. I., Herbert, P. L., S. E. and Fredrickson, D. S. A specific apoprotein activator for lipoprotein lipase. *Biochem. biophys. Res. Commun.* 39:572, 1970.
34. Lee, R. S. and Hatch, F. T. Sharper separation of lipoprotein species by paper electrophoresis in albumin-containing buffer. *J. Lab. clin. Med.* 61:518, 1963.
35. Levin, Otera, Margnambolaget AB. Personal communication.
36. Lhdygaa, F. T., Ferman, N. A. and Nichols, A. V. In: *Metabolism of lipids as related to atherosclerosis*, p. 62 (Fage, I. H., Ed.). 1965.
37. Master, A. M., Garfield, C. I. and Walters, M. B. Normal blood pressure and hypertension. Lea and Febiger Philadelphia 1952.
38. Nichols, A. V., Rehnberg, C. S., Lindgren, F. T. and Wills, R. D. Effects of oil ingestion on lipoprotein fatty acids in man. *J. Lipid Res.* 3:320, 1962.
39. Nichols, A. V. and Smith, L. Effect of very low density lipoproteins on lipid transfer to incubated serum. *J. Lipid Res.* 6:206, 1965.
40. Nikkila, E. Studies on the lipid-protein relationships in normal and pathological sera and the effect of heparin on serum lipoproteins. *Scand. J. Clin. Lab. Invest.* 5 suppl. 8, 1953.
41. Norum, K. R. and Gjone, E. Familial plasma lecithin cholesterol acyltransferase deficiency. Biochemical study of new inborn error of metabolism. *Scand. J. Clin. Lab. Invest.* 20:231 1967.
42. Oliver, M. F. and Boyd, G. S. The plasma lipids in coronary artery disease. *Brit. Heart J.* 15:347 1953.
43. Olson, A. G. Studies in asymptomatic primary hyperlipidemia. II. Clinical findings. *Acta med. scand.* 1975.1 press.
44. Persson, B. Lipoprotein lipase activity of human adipose tissue in different types of hyperlipidemia. *Acta med. scand.* 193:447 1973.
45. Quarfordt, S. H., Levy, R. I. and Fredrickson, D. S. The kinetic properties of very low density lipoprotein triglyceride in type III hyperlipoproteinemia. *Biochim. Biophys. Acta* 296:572, 1973.
46. Reaven, G. M., Juhl, D. B., Gross, R. C. and Farquhar, J. W. Kinetics of triglyceride turnover of very low density lipoproteins of human plasma. *J. Clin. Invest.* 46:1756, 1967.
47. Robinson, D. S. The function of the plasma triglycerides in fatty acid transport. In: *Lipid metabolism*, p. 51 (Horkan, M. and Stoltz, E. H., Eds.). Elsevier Amsterdam, 1970.
48. Rose, G. A. and Blackburn, H. Cardiovascular survey methods. World Health Organization Monograph, series no 56. Geneva, 1968.
49. Sata, T., Havel, R. J. and Jones, A. L. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. *J. Lipid Res.* 13 757 1972.
50. Schaefer, L. E. Serum cholesterol and triglyceride distribution in normal New York City population. *Am. J. Med.* 36:262, 1964.
51. Schrammer, V. N. and Adams, C. H. Very low density lipoproteins: Surface-volume changes during metabolism. *I. Theor. Biol.* 26:89 1970.
52. Seardcor, G. S. Statistical Methods, Ames, Iowa. Iowa Stat. Univ. Press, 1961.
53. Strong, J. P., Eggen, D. A. and Oulman, M. C. The natural history geographic pathology and epidemiology of Atherosclerosis. In: *The Pathogenesis of Atherosclerosis*, p. 20 (Winkler, R. W. and Geer, J. C. Eds.). Baltimore, The Williams and Williams Company 1972.
54. Wilhelmsen, L., Wedel, H. and Tibblin, G. Multivariate analysis of risk factors for coronary heart disease. *Circulation* 48:930, 1973.

